

nature

2 March 1978

Higher education confronts the 1990s

FOR the past few years Britain's Department of Education and Science has been wrestling with the problem of demographic change and the numbers of schoolteachers required in the 1980s. Intake into primary schools is in rapid decline at present and the number of teachers being trained has already been cut back by about 25% from its peak value of five years ago; there are more cuts to come—by the year 1981 there could be only a third of the number of teachers in training that there were in 1971. But by 1991, this decline in student population will have worked its way through to the higher education level with equally dramatic implications for the employment of new staff—maybe. It all depends on what happens to the age participation ratio, the percentage of eighteen-year-olds who enter higher education.

This ratio rose dramatically during the 1960s as new universities and polytechnics came on stream (universities comprise roughly half the higher education sector). In 1964 the ratio was 8.0%, by 1969 13.7%; since that date the ratio has hovered around the 14% mark. And where does it go from here? The Department of Education and Science has just issued a discussion document *Higher Education into the 1990s* (free from the department) which looks at policy options and predictions. The subject matter is sufficiently important that the document should be read by every one of the more than 80% of present-day higher-education teachers who will still be in employment fifteen years from now.

The decline in births has been an exclusively working-class phenomenon these past few years, but it is the middle classes that use higher education in great numbers, so to a certain extent the system is buffered against too drastic a drop; even so it is clear that, other things being equal, there will be a peak in student numbers some time in the 1980s followed by a steady fall.

Five policy options are put forward, none of them to be seen in isolation from any of the others. Two of them attempt to follow the population by responding in a passive way to the changes. First, the education system could simply expand and contract by the acquisition and disposal of staff and buildings as required. This would be expensive, particularly in the human terms of staff hired and fired—as the department fully recognises. The second version would steer higher education over the hump more economically by the use of temporary accommodation, the hiring of temporary staff, the employment of more educational technology, four-term years and so on.

The other three alternatives, however, call for more

intervention. The first would simply break with the Robbins principle that all those able and willing to enter higher education should be enabled to do so. In effect, the hump would be tunnelled through, by clamping down on expansion from now. In the 1980s many would be denied higher education, but in the 1990s it would again become more freely available. The second alternative would call for a much more varied mixture of offerings—two-year courses, part-time courses, deferred entry, more sandwich courses. The third alternative would mean much more active involvement in attempting to change the age structure and social mix of higher education so as to stimulate a new demand for higher education, which would prevent the downturn in the late 1980s. Thus there would be more positive steps to encourage participation by working-class children and some move towards providing educational opportunities later in life.

No one option is favoured in the paper, but Mr Gordon Oakes, Minister of State for Education, has been pushing a rather extreme version of the last option recently—that 50% of higher education should be reserved within fifteen years for mature students. This seems too much too soon. A major mistake of the 1960s was the establishment of so many universities in so few years—an expansion which could not be tied in to an orderly expansion of university jobs. As a result too many were hired too rapidly. There is real danger in yet another drastic change in the nature of universities—in some ways potentially more damaging as it would involve all universities, not just new ones. The objective is worthy enough, but the pace seems bound to cause maximum confusion.

But this is not to deny that higher education has a social-policy role to play alongside being a bastion of learning and a home for the enquiring mind. It is surely becoming increasingly the duty of higher education to provide an appropriate environment for less privileged members of the community—and this includes immigrants—to realise their potential. The rapid expansion in the 1960s did not adequately do that, and so we must look for slow expansion in the next ten years gradually to get to grips with the issue. This is bound to mean that some more privileged students who up to now would have scraped their way into higher education and profited little by it will have to do without; this seems no great loss—an updated Robbins principle to account for changing moods might speak, however contentiously, of students having to be able, willing and deserving in order to participate in higher education. □



Reprocessing nuclear fuel: for . . .

G. R. Bainbridge of the Energy Centre, Newcastle upon Tyne, gives his arguments for building an oxide fuel reprocessing plant in Britain

OVER the past 25 years British industry has worked towards an integrated nuclear fuel cycle for power generation. Progress continues favourably for the cycle in all main aspects:

- The centrifuge uranium-235 enriching plant at Capenhurst is progressively being commissioned.
- Consent has been given for the Central Electricity Generating Board (CEGB) and the South of Scotland Electricity Board (SSEB) to work towards the construction of advanced gas-cooled reactor (AGR) power stations at Heysham and Torness.
- Mr Justice Parker has reported on the BNFL application and it is likely that he has recommended the government to allow it probably at Dounreay.
- A UK Atomic Energy Authority (UKAEA) proposal to build a commercial fast reactor (CFR) power station, probably at Dounreay, should shortly be ready for consideration.

It is vital for national and international energy sufficiency to assure adequate reasonably-priced uranium and plutonium fuels to supplement diminishing coal, crude oil and natural gas resources; even fifty years hence it seems unlikely that other alternatives (fusion, sun, wind, waves, tides, geothermal) will be far enough developed to do this. The key to such sufficiency is used-fuel reprocessing of which Britain already has excellent large scale experience.

The United States, with almost 50% of known world low-cost uranium has a policy to defer reprocessing of used fuel rather than recover its 98% uranium and plutonium content. With great distances to transport fuel overland, no significant nuclear-fuel reprocessing industry, no CFRs and good prospects for exporting enriched uranium it may be a prudent commercial decision. Canada, with plenty of uranium and barely enough technical resources to build large-scale nuclear reactors can also get by, at least for the present without reprocessing. Both countries, however, may have severe and costly problems with deterioration of long-term stored radioactive fuel.

For Britain recycling fissile material from used AGR fuel can reduce the need for imported uranium by up to 35% or provide over 30% of extra heat from what is imported. A reprocessing plant for 1,000 tons per annum of used uranium oxide fuel can add 30 million tons of coal equivalent annually to resources, some of it to the benefit of overseas customers, with financial gain to Britain. The 1% plutonium product of the used fuel reprocessed at such a plant, blended in oxide form with about four times its own weight of uranium (not a bomb material), would provide the initial fuel of two large CFRs. At the same time the radioactive waste would have been reduced by a factor of about 50.

Confidence for peaceful co-existence between nations is an essential pre-requisite to reducing the number of nuclear weapons. Lack of such confidence in the late 1940s led the US, the USSR, Britain and France independently to produce bomb stockpiles using high enriched uranium from 'separation' plants and plutonium from elementary 'atomic piles'. Suspicion led China and India to follow. For none of those

countries were commercial nuclear fuel reprocessing plants the primary developments.

More than 30 nations have the technical capability to produce nuclear bombs and more than 100 are in agreement about the need for a non-proliferation treaty aimed at controlling the spread of nuclear weapons. These countries are willing to submit to International Atomic Energy safeguards.

At present no international sanctions are strong enough to prevent countries with modestly advanced technological capabilities from manufacturing highly destructive weapons. 'Friendly' political pressure and voluntary agreements between many nations have succeeded in reducing threat and counter-threat.

Trading is one sure way of easing international tensions. If the processors of used nuclear fuel, and their governments, assess that a country which has requested plutonium fuel will probably behave responsibly then properly supervised supply and use should be arranged whenever it is commercially practical. Britain has a long tradition in such trading.

Britain is a pioneer of nuclear wastes management. At present waste from nuclear fuel reprocessing is stored in liquid form in steel and concrete tanks and continually cooled. In the long term it would be safer to combine the valueless components of those wastes into inert solids. It has been suggested that the waste be chemically combined into glass and be stored within steel and concrete.

But it is first necessary to know what is valueless. History indicates that the wastes of earlier periods have found applications. So 'irrecoverable' disposal could be inappropriate. Caverns in stable, dry and deep salt, clay and granite formations have been suggested for disposing of such wastes. The cost will depend on several factors: whether the depository is to be on land or below the sea, near or far from the processing plant, for retrieval or not. Other suggestions have been to place the solid blocks on or under the sea bed of the deep oceans.

For this latter option, the rate of disintegration of the blocks, the rate of mixing of their contents with the near surroundings, the migration of those surrounding solids and waters need to be better known.

International agreements will have to be reached on the disposal locations and procedures. This will take time and it will be wrong to reduce the options too hurriedly when adequate temporary arrangements are feasible.

A reprocessing plant for throughput of about 1,000 tons per annum will cost in excess of £500 million, including radioactive waste storage, some R & D expenditure (say 5% of the total) and a decade of operating costs (perhaps 25% of the total). That figure would be increased if extra cooling ponds capacity is needed and a decision is made to go forward with glassification for ultimate disposal. But the uranium alone in the 3,000 tons of used AGR fuel sent for reprocessing up to 1995 would be worth in the region of £200 million and the plutonium (say 30 tons) very much more. Introduction of a similar amount of overseas fuel processing business would completely recover the costs of the plant.

The plant costs are of the same order as those for one AGR power station and the reprocessing service from it is likely to provide for upwards of twenty such power stations. The uranium recovered could reduce imports of fresh uranium for them by 15% and the plutonium could fuel some 30 CFR power stations.

Apart from reducing the amount of radioactive wastes for disposal, there are advantages for the balance of payments and the ongoing nuclear power programme to be gained from reprocessing. □

... and against

Czech Conroy, Energy Campaigner for Friends of the Earth in the UK, argues that an oxide fuel reprocessing plant should not be built

REPROCESSING—the extraction of plutonium and uranium from nuclear fuel elements after they have left the reactor—originated as a key step in nuclear weapons programmes. The initial financial and intellectual commitment to the technology then resulted in nuclear weapons states, and some other countries, regarding reprocessing as a desirable—if not an inevitable—step in all radioactive waste management. (Interestingly Canada, which is not a nuclear weapons state, has no plans to reprocess its spent fuel and has never regarded reprocessing as necessary or desirable). In October 1976 the United States followed Canada in this respect when President Ford announced: “I have decided that the United States should no longer regard reprocessing of used nuclear fuel to produce plutonium as a necessary and inevitable step in the nuclear fuel cycle [and that] avoidance of proliferation must take precedence over economic interests.” In the same year British Nuclear Fuels Ltd (BNFL) applied for planning permission to build a Thermal Oxide-fuel Reprocessing Plant (THORP) at Windscale in Cumbria, and at the resulting inquiry into their application they argued that reprocessing was necessary and desirable for radioactive waste management.

To date UK experience of reprocessing has been confined almost exclusively to uranium metal fuel, either from the military programme or from the Magnox reactors which formed the first phase of Britain's civil nuclear programme. The cladding of this fuel is a magnesium alloy which corrodes significantly after two years' storage in water, leading the Royal Commission on Environmental Pollution (RCEP) to conclude in 1976 in its report *Nuclear Power and the Environment* that “There appears to be no real alternative to the reprocessing of Magnox fuels . . .”. By 1977, however, Sir Brian Flowers, former chairman of RCEP, had come round to the view that Magnox reprocessing might not be necessary since Magnox fuel can also be stored in gas before disposal—a much more corrosion-resistant environment than water.

The fuel elements which THORP would reprocess would be clad in more corrosion-resistant materials than Magnox fuel—stainless steel for British oxide fuel elements and a zirconium alloy (zircaloy) for foreign fuel elements. Nevertheless, BNFL argued at the Windscale Inquiry that the corrosion of stainless-steel-clad fuel elements might be high if the elements were stored in water for more than ten years. Objectors to THORP argued that improvements in the water chemistry of storage ponds would reduce corrosion levels, and that like Magnox fuel elements the stainless-steel-clad ones could be stored in gas.

The United States plans to store its spent fuel for several years while it decides whether or not to recover the plutonium and uranium. If the spent fuel were not reprocessed it would be put in overpack canisters and disposed of in a geologic repository. It is sometimes argued that, since the fuel elements would contain all the radiologically hazardous plutonium produced, it would be preferable to reprocess the fuel and recycle the plutonium in thermal or fast reactors.

This argument, however, overlooks the fact that the thermal or fast reactor fuel containing the plutonium must also be disposed of ultimately, and when these and other wastes are taken into account the amount of plutonium to

be disposed of under the reprocessing route is only between five and ten times less than that under the direct disposal route. Since “recycling of plutonium requires reprocessing, mixed oxide fabrication, and management of various additional types of waste, all of which raise serious questions of economics, health and safety, and environmental matters” (*Nuclear Fuel Cycles and Waste Management*, p27 New York; American Physical Society, 1977), many people believe that waste management options involving reprocessing do not reduce the radiological hazards of nuclear waste.

Another argument advanced in favour of reprocessing is that the uranium and plutonium which would be recovered will be essential in the future if we are to meet official forecasts of energy demand. These forecasts have been criticised at the Windscale Inquiry and elsewhere on simple matters of principle. Work carried out at a variety of research establishments in the UK and elsewhere suggests that a combination of conservation and renewable energy technology systems can substitute for oil and gas more rapidly and cheaply than plutonium-fuelled fast reactors, thereby eliminating any need for plutonium as a fuel. In any case, deferral of a decision on THORP need not affect feasible fast breeder programmes since by 2000 a further 50 or so tonnes of plutonium could be extracted from Magnox fuel.

Although oxide reprocessing is not particularly attractive either from the standpoint of reducing radiological hazards or providing fuel, its supporters claim that it could have economic advantages over the alternatives. But oxide fuel reprocessing has not been demonstrated on a commercial scale anywhere in the world, and its estimated costs have soared rapidly during the last few years so that the United States government now doubts whether it is economically viable. Nevertheless, if BNFL's cost estimates are accurate, reprocessing nuclear fuels through the THORP plant and recycling the plutonium and uranium will almost certainly be cheaper than storage and direct disposal of the spent fuel, since foreign customers are prepared to sign very unfavourable contracts to get rid of their spent fuel. Uranium prices would have to fall well below \$30/lb before it would become cheaper to buy fresh uranium than to recycle uranium and plutonium. The price of uranium will be influenced to a large extent by uranium requirements and hence future levels of installed nuclear generating capacity—so that as forecasts of nuclear capacity rapidly decline the relative economic advantage of reprocessing is also likely to decline. A case can be made, however, for proceeding with THORP on economic grounds.

Against the probable economic benefits of THORP must be set its external costs. Outside the area of radioactive waste management these costs relate primarily to the possible use of plutonium as a nuclear explosive, particularly by national governments. Increasing recognition of this risk has resulted in the establishment of the two-year International Fuel Cycle Evaluation programme (INFCE), in which Britain is participating. The primary function of INFCE is to identify technically and economically feasible technologies which are more proliferation-resistant than the kind of oxide reprocessing BNFL is proposing to carry out. Meanwhile, there is a belief that the construction by Britain of a plant for the separation and distribution of nuclear-weapon material would be politically catastrophic.

The relative weights to attach to any economic gains from oxide reprocessing, its ultimate impact on the fabric of society, and the possibility of it increasing the risk of nuclear war are a matter of political and ethical judgment. Like President Ford, I believe that “. . . avoidance of proliferation must take precedence over economic interests”. We shall soon see whether or not the British Government believes that too. □

Czech Conroy's book What Choice Windscale? The Issues of Reprocessing, is published by Friends of the Earth and the Conservation Society (February 1978, price £1).

Navy lifts veil on PWR research

The PWR is not new to Britain.

David Fishlock describes Rolls Royce's experience in developing the reactors for the Navy

HMS SCEPTRE, Britain's 14th nuclear submarine—the first to take a name not already made famous as a capital ship—was commissioned by the Royal Navy last month. The event coincided with a decision to lift the veil slightly on an R & D programme which has remained secret for nearly two decades.

The peaceful application of the technology which has transformed the submarine from a submersible of strictly limited endurance into an underwater fighting ship has long been a matter of serious discussion. Several factors have inhibited progress. Costs of marine nuclear-propulsion-systems have remained high enough to limit interest to very large vessels or special-purpose craft such as icebreakers, submarine oil transports, or submersibles for seabed construction projects. The world has also been slow to develop universally acceptable safety criteria for seagoing nuclear steam-supply-systems that would allow nuclear vessels to steam freely and gain ready access to ports.

A third factor has been the military origins of much of the development of marine reactors, and the difficulty of separating a civil product from a highly classified technology. This has been an important factor in Britain, where the submarine reactor programme has its origins in an Anglo-US treaty signed in 1958. Under its terms the US Navy provided Britain with technology to advance the Ministry of Defence's embryonic project at Harwell by several years. Britain procured a reactor from Westinghouse Electric for HMS Dreadnought, its first nuclear submarine, and also received a "technology transfer" relating to the Dreadnought PWR technology at that time.

But a new factor has recently asserted itself. The electricity supply industry has persuaded the government that it still has enough worries about the thermal reactor system on which its nuclear construction programme depends, namely the advanced gas-cooled reactor, to justify pursuing an alternative system. This is the Westinghouse pressurised water reactor (PWR), developed from the company's submarine reactor to a thermal megawatt output about 40 times as great. Rolls-Royce, the company chosen to manage the Navy's submarine reactor programme, believes that it has technology and experience which could be valuable in developing a 'British PWR' for electricity generation, as well as

surface vessels such as the icebreaker Canada is proposing to cleave through 10ft-thick sheets of ice.

Vice-Admiral Hyman G. Rickover, responsible then (and still) for the US Navy's nuclear fleet, had asked that Rolls-Royce should be the company to manage the procurement and technology transfer for Britain. He chose it because its engineering and quality assurance standards were closest to those he had found essential for the success of the highly sophisticated nuclear engine. Techniques for programme management, planning, and financial control were more backward in Britain in the late 1950s, but the Westinghouse/Navy methods were all part of the technology package the US was willing to transfer.

Britain's first PWR

To receive it, the Ministry of Defence encouraged the establishment of an industrial consortium called Rolls-Royce and Associates, comprising 54.1% Rolls-Royce, with Babcock and Wilcox (which came in in 1967), Foster Wheeler and Vickers each having 15.3%. Its initial tasks were to arrange for the procurement of a US-built nuclear steam supply system (NSSS), the Westinghouse S5W PWR, for Dreadnought; and the accompanying technology transfer that would enable it to complete the design and assemble Britain's first PWR. This was built on the north coast of Scotland, adjoining the site of the Dounreay Fast Reactor experiment. It was incorporated into about 2,000 tons of submarine, constructed by Vickers, including the entire propulsion system of a nuclear submarine. (Steam propulsion, it may be recalled, was then something new to submariners.) Later named HMS Vulcan—after the lame god who never fought—the half-submarine operated as a prototype NSSS, and also as a development and training facility where new concepts could be tried out, or operational troubles simulated. This British PWR has been running since 1965, during which time it has been extensively modified in two major refits to adapt it to developments from the design and development department of Rolls-Royce and Associates.

From an initial team of 200, the company has grown to 1,300 today. It has provided the Navy with 16 PWRs and 30 cores. As it learned from Westinghouse and then won the confidence of the Navy, the Ministry of Defence delegated to it more and more

responsibility. "We taught ourselves the fundamental technology that was not part of the package the government bought from Westinghouse," says Peter Goodwin, its managing director. Since 1965 it has managed HMS Vulcan for the Ministry of Defence under the direction of a Captain Superintendent who has responsibility for health and safety at the facility. Thus it is the only private company in Britain operating a power reactor. About 300 of its staff are based at HMS Vulcan.

All but a handful of the other 1,000 staff are based at Derby, and about 450 of them are engaged in research, development and design. The Ministry of Defence has delegated to the company the design authority for the NSSS "from cradle to grave", says Peter Jones, the director responsible. But he works closely with the Navy's Ship Department at Bath; with HMS Vulcan (managed by a fellow director); and with Harwell. The company's adviser on safety is the Safety and Reliability Directorate at Risley.

The R & D programme arises from consideration of four pressures upon the company as an MOD contractor, says Jones. First, it arises from Navy requirements for improvement in performance of future submarines, and their testing in prototype. Second, it arises from the need to apply innovations to the first of any new class of boat, or their "backfitting" to existing boats. Third, it arises from problems encountered at sea or in prototype operation. Fourth, it arises from new thinking about reactor design of safety criteria.

The company has been responsible for two major advances in the performance of the first British PWR, one already in fleet service since 1973, and the second scheduled to enter service in the near future. It is now developing the reactor for Britain's submarines in the 1990s—a programme that owes much to the fourth consideration.

The focal point of reactor development in each case is the core, the very high precision assembly of fuel and control mechanisms that forms the heart of the NSSS. Even excluding the cost of the highly enriched uranium fuel itself, the core accounts for 23.5% of the price paid by the MOD for the reactor. But core design also intimately affects every other feature of PWR design.

Increasing endurance

The company's first development task, started in 1960, was for a more powerful, longer-lived core. Endurance rather than additional power is the over-riding objective of submarine reactor development, because fuel changing necessitates a major refit, including

cutting through the pressure hull to access the reactor compartment. The new core first went critical in HMS Vulcan in 1968. It raised more power, and operated more than half as long again, as the first British-made core modelled on the US design. The new core entered service with the fleet in 1973. It can be backfitted to all UK submarines except Dreadnought.

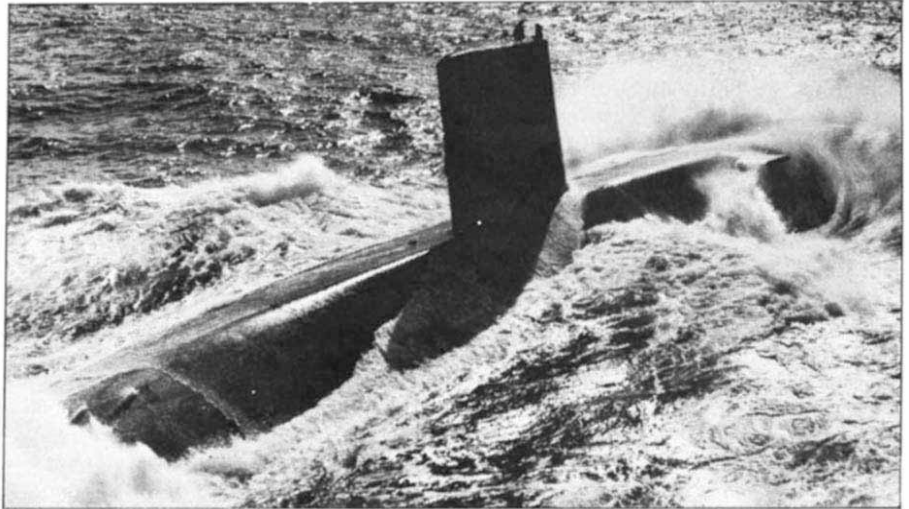
In 1968 Rolls-Royce and Associates received its second major development contract from the Navy, for a reactor of still longer endurance between refuellings, combined with a new characteristic of fast-increasing importance—less noise and vibration. Power level, however, was to remain almost unchanged. Concurrently, the company set up a team to investigate alternative reactor configurations, only to conclude that the Navy's specification could best be met with a third-generation British PWR. This core first went to power in HMS Vulcan last March. The Navy believes it will prove to have an energy output over its lifespan several times greater than the first British PWR.

Jones estimates that each of the steps in reactor development has been achieved for an outlay of £6 to £7 million, excluding the cost of prototype testing. The second-generation core achieved its advantages for a 19% increase in application cost over its predecessor; the third-generation core for between 16% and 30%, depending upon whether one costs plant hardware to the core required or to the need for routine replacement. These figures are plainly indicative of a highly cost-effective defence research programme.

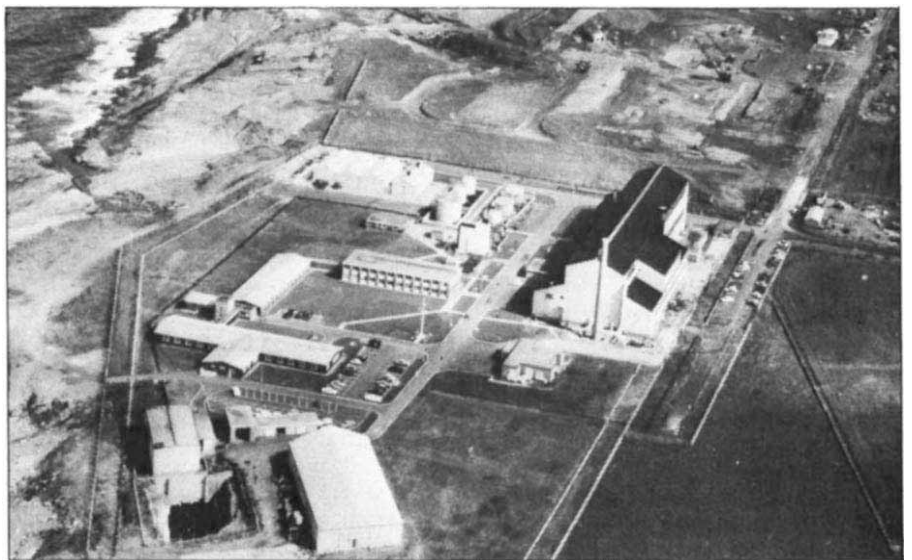
Research resources at Derby include Neptune, a zero energy research reactor begun in 1962, for physical tests on fuel modules. The fuel design has been considerably modified in detail for resistance against shock and the thermal stresses. The big gains in performance derive from the way it is made, assembled, cooled and controlled.

Hydrodynamic studies on cooling the core are carried out with perspex scale models of the pressure vessel and core, about one-third full scale, illuminated by lasers to reveal the flow patterns around the densely packed fuel. Larger rigs permit testing of full-scale components and sub-assemblies for heat transfer, noise, and so on.

The company has mounted a major development programme for non-destructive testing of critical reactor components such as the steel pressure vessel. This vessel has to be much closer to the dimensions of that of a large power-station PWR than its power output suggests to meet the Navy's specifications for military ro-



HMS Valiant surfacing off the west coast of Ireland



HMS Vulcan: the Royal Naval Test and Training Establishment, Dounreay, Scotland

bustness. Thus it is about two-fifths of the height, one-eighth of the weight and half the thickness of the vessel specified for a 1,300 MWe PWR—for one-fortieth of the rating. Studies have included 100% ultrasonic inspection of critical parts of the pressure vessel of the longest serving Navy reactor, at HMS Vulcan, by a scientist lowered into the vessel in a "lead bucket". The last word on the condition of this vessel will not be written, however, before 1982, when the prototype is dismantled and a *post mortem* held on such parts as the pressure vessel, which by then will have accumulated about 18 years of irradiation experience.

The opportunity to carve up this vessel will come as a result of a recent decision by the Navy to build a new shore test facility at HMS Vulcan. The Controller has placed a design and development contract with Rolls-Royce and Associates for the reactor it wants for service in the 1990s. The first step will be to test a new reactor built alongside the present facility, construction of which is scheduled to

begin next year, for completion in the mid-1980s.

This will be another PWR. Enthusiasts among some US nuclear designers for lightweight, more powerful submarine reactors may misunderstand Navy requirements, says Jones. The Navy has no special interest in paring weight to the bone and needs to optimise the conflicting requirements of power and reactor noise. But the company is coy about the performance expected of the new PWR: "we'll end up with a safer, more reliable plant to meet a more stringent set of military objectives".

Nonetheless, there will be some big differences about the new test facility. For one thing, it will be the first PWR the company has designed "from scratch"—previously it has always been adapting the S5W Westinghouse reactor. For another, the plant will be laid out quite differently: the plant layout will be "exploded" for ease of access. Only the reactor itself will be contained in a representative section of pressure hull. □

University safety report hushed up?

THE findings of a study by a senior inspector of the UK Health and Safety Executive (HSE) into health and safety in British universities may be controversial. Since the report of the study was submitted to the HSE last summer, it has not been made available to any of the organisations concerned with health and safety in universities. Neither the Committee of Vice Chancellors and Principals, nor safety officers in universities, nor the unions representing university employees have seen the report, even though they had all been led to believe that it would be available by the end of the summer of 1977.

The HSE puts the delay down to the length of time it has taken to consider the report and produce its own recommendations. But the general feeling outside the HSE is that the delay indicates that the report is embarrassing to someone. The unions, for example, feel that it may recommend major improvements to safety standards in universities which could cost of the order of £40 million.

The purpose of the study was to identify health and safety problems peculiar to universities. Prior to 1974, when the Health and Safety at Work Act was introduced, university employees had not been covered by health and safety legislation, so the HSE felt that a study to pinpoint special problems would be helpful to its inspectors (who were accustomed only to the problems encountered in industry). Hospitals, schools and other new entrants under the 1974 act have also been subjected to similar studies.

The university study was conducted by Miss N. Curry, of the Scotland West Area Office. She visited six universities chosen by the HSE in consultation with the CVCP—Leeds, Strathclyde, East Anglia, Cambridge, Swansea, and Salford—where she consulted heads of departments, union representatives and workers on the shop floor. By the Autumn of last year she had submitted her report to the HSE.

In contrast to the union's view that safety standards in the universities will

have to be brought up to those in industry, the CVCP thinks the report is likely to suggest that special provision must be made for universities and research establishments, in accordance with the recommendations of the Robens Committee on Safety and Health at Work. In the CVCP's view a major problem in subjecting universities to the same health and safety requirements as industry is in determining the rights and obligations of students.

Both unions and university safety representatives, however, are anxious to see the report as soon as possible. The CVCP wants to revise its code of practice on health and safety and the unions are anxious to advise their members in the light of the report's recommendations. In a reply to a letter in the new year from John Akker, Deputy General Secretary of the Association of University Teachers, Bill Simpson, Chairman of the Health and Safety Commission, said that the report would be available sometime in the Spring. As yet, however, the HSE can give no clearer indication of when that will be.

Judy Redfearn

South Korea to raise investment in science and technology

SOUTH Korea is planning major expansions in the development of science and technology. According to Choe Hyong-Sop, Minister of Science and Technology, the investment in science and technology is to be increased to 1.5% of the GNP by 1981 and 2.5% by 1991. The role of the Korean Science Institute in the development of the country's technology is to be expanded, and a vocational college established.

In a recent briefing session with President Pak Chong-hui, the Science Minister explained that the basic directions for technology policy are the "strategic development" of industrial technology and the "deepening of an atmosphere conducive to the development of science and technology". Considerable emphasis is being placed on the adaptation of advanced foreign technology to the needs and conditions of Korea, the attraction of private investment into the development, the training of technical manpower and the liberalisation of technology imports.

These aims are reflected by other Ministries. At last January's trade promotion conference, the Foreign Minister Pak Tong-chin said that efforts would be made to diversify sources of capital technology imports to Europe and the Middle East oil states. The Ministry of Commerce and Industry is simplifying the procedures and regulations governing the intro-

duction of foreign technology. Concentrated support is to be given to a number of industries, including the chemical and metal-working industries; and several other industries, including electronics and the motor-vehicle industries, are to be developed into major export industries. Large enterprises will be encouraged to establish their own technological research institutes, and during the current year the Ministry will release \$494,000 from local currency loans and \$1,300 million from foreign exchange loans for laboratory equipment for such institutes.

Behind all these plans for expansion, however, one can sense a growing concern with energy supplies. South Korea is presently a coal producer, but importing of coal may become necessary as early as 1979. Oil is a major import, both for transport fuel, power generation, and for use in the important chemical industry. Plans are going forward for a new petrochemical

complex—the country's third—and new price stabilisation measures for essential commodities which should help hold down prices of certain types of oil and petroleum products. New sources of oil supplies are being sought in Africa and South America, in addition to the current Middle East suppliers (Iran, Kuwait, Saudi Arabia), in the hope of maintaining stable supplies and prices.

At the same time, the Energy and Resources Minister announced that no more oil-burning power stations will be undertaken; in future all new stations will be either lignite-burning, nuclear or tidal. In support of this policy, testing facilities for nuclear fuel processing with an annual uranium capacity of 10 tons are to be set up this year at the Taedok Specialised Technology Research Institute, and testing operations will commence by the end of the year.

Vera Rich

Refusnik challenges withholding of his degree

SINCE 1976, academic brilliance has been a necessary but not sufficient condition for receiving a higher degree in the Soviet Union—a proper political outlook is equally, if not more, important. According to Article 24 of the regulations of the Higher Qualifications Commission (*Vyshshaya Attestat-*

sionnaya Kommissiya—VAK) which assesses a university's recommendation that such-and-such person be awarded the degree of Candidate (PhD) or Doctor, it is required that such persons have shown themselves to be among "those who follow the norms of communist morality and those who act

according to the principles of Soviet patriotism and proletarian internationalism”.

These regulations are by no means simply a paying of lip-service to Party and ideology. In what promises to be a test case, *refusnik* Arkadii Tsinober is challenging the right of VAK to withhold his Doctor's degree on the grounds that he is “amoral and unpatriotic”.

Tsinober, who had worked at the Institute of Physics of the Academy of Sciences of the Latvian SSR for 15 years, defended his Doctoral thesis in the summer of 1975. Shortly after-

wards, he applied to emigrate to Israel. At this time, although the new regulations of VAK had not come into force, their content was well known in Soviet academic circles, and dissidents were predicting that by 1985 no non-Party person would be able to hold a major academic or scientific post.

Tsinober's application for a visa was turned down on the grounds of the “secrecy” of his work on MHD—an excuse which might have appeared more valid had not his colleague and co-author Professor H. Branover been allowed to emigrate. He never receive his Doctoral degree—and this,

he claims is solely due to his desire to leave the country. Since the Soviet Union is a signatory to the Covenant on Civil and Political Rights, which guarantees the right of the citizen to leave any country, including his own, Tsinober claims that the refusal of his degree is unconstitutional, and is attempting to make good his claim through the Latvian courts.

In a recent message, he stressed that it is not simply a paper qualification that is at stake—that is a minor matter—but the whole question of state intervention in the academic process.

Vera Rich

New science policy minister for the Netherlands

AFTER a record breaking period of 206 days without government, the Christian-democrats and conservatives under Dr Dries van Agt as prime minister, finally came to power in the Netherlands at the end of last year. The new minister for science policy is Dr Rinus Peijnenburg, the right hand man of Dr van Agt during the days without government.

Formulating science policy will be a new area of government to Dr Peijnenburg. As well as being minister for science policy, he is acting as economic adviser to Dr van Agt, who unlike most prime ministers is not an economist. Even though he has little knowledge of science and technology, Dr Peijnenburg's varied experience as a politician should prove useful in his new post.

During the formation of the new cabinet last year it was doubtful that the position of the coordinating minister for science policy would be strengthened. Recently, however, Dr van Agt has indicated that the science policy minister is to have more power and more money for running his department.

The organisation remains the same as in the previous cabinet. Responsibility for science policy lies with the department of education. Two ministers have some responsibility for science: the minister for education, who is concerned with science education in schools and universities, and the minister for science policy whose concern is science outside educational establishments.

With his other commitments, it remains to be seen how much time Dr Peijnenburg will spend on science policy. After a couple of months in office, however, he is taking his responsibilities for science very seriously and he may not have much time for his duties as economic adviser. He already has a science budget for 1978 which was allocated by his predecessor, Fokele Trip, who had been in charge

of science policy for four and half years. The total budget for 1978 is 5.9 billion guilders of which 2.8 billion is to come from government funds (1.2 billion is to be spent in universities). Industry — mainly the five multinationals, Philips, Shell, Unilever, DAM and AKZO—will spend 3.1 billion. The percentage of the gross national product spent on the total scientific effort will remain at 2.1%, the same as in 1977. This is the greatest percentage for civil research in the EEC after West Germany.

Dr Peijnenburg's science policy will follow along the same lines as that of Fokele Trip. Like Mr Trip, he will

encourage technological innovation in small-to-medium-sized industry and has even promised a memorandum on innovation within a year. Other new activities include the setting up of a group to look into ageing research and a committee is to be set up to look into the state of biochemical research in Holland. A special group, linking various departments, will be established to advise the government on Dutch policy for space research and technology in the 1980s. In 1978 a decision will also have to be taken on the development of an extended version of the Fokker F-28 passenger plane at a cost of 200 million guilders. **Casper Schuurings**

Biowarfare lab goes civil

The Ministry of Defence's (MOD) Microbiological Research Establishment (MRE), Porton Down, is to be retained for civil research after 1979, the government announced last week. The illustration shows a category IV containment facility for microbiological research at Porton.

The future of MRE has been uncertain for more than a year. In the 1976 Defence White Paper, the MOD announced its intention to shed its responsibility for the establishment—where it has carried out most of its microbiological warfare research.

Last week, however, in reply to a question on MRE's future in the House of Commons, Dr John Gilbert, Minister of State at the MOD, said that the government had “decided to retain MRE as a civil establishment subject to satisfactory administrative arrangements being made” because it had “concluded that many of the capabilities and facilities of MRE represent a national asset of value”.

Before “satisfactory administrative arrangements” are made, however, the MOD will continue to “manage the establishment in 1978–79 and reduce it

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to the size appropriate for the civil programmes now foreseen. This is expected to be about two-thirds of present strength”. In the meantime “proposals for the transfer of responsibility from the MOD to the Department of Health and Social Security and the Public Health Service are under discussion”.

Research in third world countries: Pugwash plans controls

PUGWASH, the international organisation of concerned scientists usually associated with nuclear disarmament, set up a workshop in Delhi in January to resolve an urgent new problem: the relationship between researchers in the first and third worlds. Feeling has been building up in developing countries over the past eighteen months against collaborations between the rich 'northern hemisphere' and the poor south—where the north provides most of the money and consequently determines the research priorities. Such research is always to the long term benefit of the north and to the disbenefit of the south, in the third world view.

Dr Geoffrey Oldham, deputy director of the Science Policy Research Unit (SPRU) at the University of Sussex, told a Sussex seminar last week that the feeling generated by the perceived or misperceived wrongdoings of the north is so strong that India, for example, had practically closed up shop on such collaborations.

So Pugwash, shifting towards an interest in science in developing countries, has stepped in to try to set up 'guidelines' for such research that would allay third world fears.

The fears centre on eight main issues, Dr Oldham said. The first is scientific colonialism: that first world researchers exploit cheap research labour, or lax safety and health regulations, to do their own research abroad under the pretext of collaboration. The third world scientists involved are left untrained, and when the visiting team withdraws it takes its data with it.

The second concern is that since most of the collaborations are funded from the north, and since the north determines the nature of the research, the north is making a 'de facto' determination of the science policy of the south.

The third fear is a more common one: that the north is exploiting the south commercially through its research. An example, said Dr Oldham, was research in Lagos on the conversion of the cassava root to gari, a flour-like West African staple. The traditional process takes the village housewife five days of hard labour, and Lagos had been working on the physics and biochemistry of the conversion in an attempt to speed it up. Lagos solved the scientific problems, but balked at the engineering necessary to turn their discoveries into a practical process. So a Bristol firm moved in "under the

guise of scientific collaboration", dealt with the engineering difficulties, and promptly claimed world patent rights. Most of the fruits of the work have therefore accrued to the firm, claimed Dr Oldham.

The fourth problem is that most collaborations have failed to stimulate the growth of scientific and technical capabilities in the third world host country: there has been no transfer of expertise.

The fifth has been raised by the "several cases" where pharmaceutical companies have instituted drug trials on developing country nationals under conditions that would have been forbidden in the north.

The sixth fear is that one of the objectives of collaboration is to discover bright researchers in the developing countries and to cull them for research in the north—or whether that is an objective or not, that the result of such collaboration is often the loss of the best talent from the country.

Next is the observation that of the total research effort in the north only a tiny fraction is available for collaboration with the south; more than 50% of the north's research is under military wrappers, and a further substantial fraction controlled by the constraints of industrial secrecy. So seen as an effort at the transfer of expertise, it is at best very partial.

The eighth and last concern is the one that brought affairs to a head: the fear, generated by the 'discovery' of two such cases in India, that collaborative agreements made ostensibly for non-military reasons are in fact being conducted to further the military ad-

vantage of the north. The first of these cases was the celebrated issue of the impotent mosquito, a programme launched by WHO to introduce floods of impotent males into the mosquito population and so control the development of the mosquito-borne parasites of filaria, dengue fever, and malaria. The programme concentrated on the species responsible for filaria and dengue, and neglected the more prevalent malaria (which at the time was coming under the control of DDT). The Indian press claimed that the real objective of the research was the investigation of biological warfare using dengue fever.

At a time when malaria was on the increase again the press charged that: the priorities of the research were not consistent with Indian needs; that the chemical sterilisers used were very toxic in the environment, and potentially dangerous; that their use would have been disallowed in the US; that possible mutations could lead to more dangerous strains; and that the concern of the research with the use of dengue in biological warfare was "quite blatant".

WHO responded with its own explanation, but whatever the rights and wrongs of the case the seed of distrust had been sown. And it was germinated by the discovery that the Bombay Natural History Society's collaborative project on the study of migratory birds as a carrier of disease was funded by the Migratory Animals Pathological Survey—a branch of the US Army. Migratory birds and other animals, which follow very predictable paths, would be very effective carriers of biological warfare agents.

There thus arose a tremendous backlash in India against collaboration, leading to what Dr Oldham called "almost a complete closure of doors". This nervousness spread around, said Dr Oldham, and has led to most developing countries setting up some government agency to assess collaborative research projects.

In this atmosphere, and with a view to making a presentation at the 1979 United Nations Conference on Science and Technology for Development, a Pugwash workshop met in Delhi in mid-January to see if they could agree to a set of 'guidelines' to inform and control future collaboration. They could not—though they produced a draft set and instructed the Indian group to complete it, for initial submission to a four-man 'editorial board' and its ultimate adoption by the Pugwash council.

Agreement was reached that the guidelines should not extend to commercial agreements, a vexed area which Pugwash was happy to leave to the forthcoming UN Conference on



"It started out as joint research into textiles!"

Technological Cooperation among Developing Countries (TCDC). On the science, said Dr Oldham, the meeting took "a very positive approach". Whereas it could have become a forum for recriminations, looking only for controls on the negative effects of collaboration, the workshop took pains to find ways of enhancing the benefits.

The workshop recognised that it was developing guidelines in an area which was relatively little researched. "We had to go by anecdotes," said Dr Oldham. SPRU's own 'Project Perseus', which had looked at the effects of international collaboration among OECD countries (principally Europe, America, and Japan), had run into a problem in defining 'success', and had been unable to come to many policy recommendations—except to point out that the more basic the research, the more successful the collaboration.

Beyond that, some work in Thailand had shown that the most highly regarded research had involved long-period collaborations.

The rest lay with hearsay and experience, said Dr Oldham, though he was inclined to accept the views of one of the most experienced members of the workshop—Dr Nayudamma, sometime head of both the central leather research institute, Madras, and India's CSIR. Nayudamma criticised the international organisations such as FAO, UNIDO, ILO, UNESCO, WHO, UNEP, and ESCAP, who he said had all tried at one time or another to claim leather as their territory, all, said Nayudamma, "fishing for projects". Success came, according to Nayudamma: when there was clear understanding of the purposes of the collaboration on both sides; when the main objective was the active building

up of local expertise; when there was trust and friendship at the top; when statuses were equal; when consultation was continuous; and when the research was more basic than applied.

"As a researcher," said Dr Oldham, "I've felt the need for information. The anecdotes might be aberrations. It looks like an interesting topic for further research. But the developing countries have a very considerable concern about the experience of collaboration. All of us must take this very seriously".

Robert Walgate

Nature would welcome hearing from scientists, particularly in the third world, whose experience of scientific collaboration between the first and third worlds might shed light on this issue

A RECENT visit to eastern Asia allowed me to see something of the pollution problems in several countries. During the last twenty or so years, since the world-wide public interest in the environment has developed, Japan has been castigated as the prime example of the danger of pollution from rapid industrial growth. Every text in this field describes horrific air pollution in Tokyo and other cities, as well as the tragic mercury poisoning at Minnemata.

My first impression of Tokyo was one of surprise; the air seemed as clean as that of London. This impression was confirmed when I visited the Environmental Agency, and met Dr Michio Hashimoto, Director of the Air Quality Bureau. I saw the records which showed that up to some ten years ago the air in the Japanese cities deserved its reputation, but that since then the improvement had been spectacularly rapid. Levels of smoke and soot are now as low as in most western cities, and those of sulphur dioxide are lower.

This improvement is the result of vigorous government action, with scientists like Dr Hashimoto in the van. Up to the mid nineteen sixties, he said "Japan had been heaven for polluters". Now this had changed, and "For the last ten years, it has been hell". The earlier period had been that of the 'Japanese miracle' of unprecedented economic growth, when the environment had come a bad second. However, those who said that growth and pollution must always go hand in hand have now been shown to be wrong. It is true that the rate of growth has slowed down somewhat, but the process has not been reversed. The clean air of today exists with substantially greater industrial production than existed with the foul air of ten

years ago.

Although Japan has not solved all its problems, the impressive new Institute for Environmental Studies, with facilities most scientists in other countries would envy, demonstrates their intention to prevent anything like a

Heaven and Hell

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KENNETH MELLANBY

second Minnemata. Regulations are strictly enforced, and controls are costing industry a great deal of money. However, it is realised that this may sometimes be a good investment. Quite apart from the loss of life and the misery caused to the afflicted, it now appears that the sum that has been paid in compensation to the Minnemata victims is astronomically greater than it would have cost to contain the waste mercury discharged by the industry.

Conditions today are somewhat dif-

ferent in South Korea and Taiwan. In both these countries industrial growth of the kind previously seen in Japan is taking place, but the programme of both growth and of environmental amelioration is ten years behind. Pollution control is not yet a priority, though the authorities are now becoming aware of the problem, and I expect they will soon follow Japan's good example. But they have not yet started to do so. In Taiwan, on the edge of the booming city of Taipei, just beyond the new and magnificent Grand Hotel, rises the mountain of Yang-min Sang. This was once noted for the purity of its air, one reason why before dawn hundreds of Chinese trek upwards to sites where they perform Tai Ch'i Chuan, Taikwondo, Karate and other exercises and martial arts practised to develop mental and physical fitness. Unfortunately the devotees are not getting the benefit of pure air, for this today is much more polluted than that of central London—or of Tokyo.

Some European environmentalists are reluctant to believe that the conditions in Tokyo are so improved. They produce photographs of the citizens in the streets wearing masks over their faces, which they say is done because of the dirt in the air. This suggestion amuses the Japanese, who actually are only being socially responsible. They wear masks when they have a cold and do not wish to infect their colleagues. They have taken the transmission of infection more seriously than we have in Britain, where the war time slogan 'Coughs and sneezes spread diseases' seems to have been forgotten, judging from the behaviour of many passengers in public transport who make little attempt to keep their germs to themselves.

correspondence

The health of physical anthropology in Britain

SIR,—It was with some surprise that we read Bernard Campbell's remarks on the state of health of British physical anthropology under the title "Is British physical anthropology dying?" (19 January, page 196). In other circumstances, a doctor who is unable to answer that question could easily find his competence doubted, particularly if he has not properly examined the patient!

Physical anthropology has been faced with the same financial limitations that have affected all other university departments. Certainly many academics have left for posts abroad, but those who have been appointed to posts in Britain have attracted support for their work.

Dr Campbell suggests that anatomy departments should no longer be the "primary resource" for human palaeontology—we disagree. It is our view that anatomists with human and primate dissection experience are still those best fitted to examine, describe and interpret fossil hominid material. Indeed, turning to Britain today, much of the exciting new fossil hominid material that is being recovered in East Africa is undergoing analysis in our London departments, including all of the Koobi Fora (formerly East Rudolf) hominid remains and a substantial proportion of those from Olduvai Gorge as well as material from another site that is, as yet, unannounced. It may be that this wealth of material is stretching the capacity of those entrusted with its study, but it does not support the conception that human palaeontology in Britain is even poorly, let alone dying.

M. H. DAY

*St Thomas's Hospital Medical School,
London*

B. A. WOOD

Middlesex Hospital Medical School,

SIR,—We read with interest Dr Bernard Campbell's observations on our subject and on our department. We agree with some of his comments and we share his hopes for the future of physical anthropology in the United Kingdom. However, we feel that the overall tone of his writing reflects an imperfect understanding of the nature of the modern study of human evolution. Furthermore, it contains a number of important misunderstandings that may be due simply to his long absence from British academic life.

The main issue on which our views diverge from those of Dr Campbell is the question of what properly constitutes the study of human evolution. Dr Campbell places evolution predominantly in the past and claims that "human palaeontology" is the "most fundamental branch of the science of physical anthropology". This is clearly absurd. The biased selection of skeletal material which has come to us from the past is a necessary source of data on human variation over time. Similarly, biological data from contemporary populations is the source of information on human variation in geographical and social dimensions.

Neither source however, is of the

slightest value without a central, theoretical framework within which the data can be interpreted. The fundamental framework of physical anthropology is evolutionary theory and the proper development of this requires several different kinds of study that are not merely ancillary to human palaeontology. The key to a successful evolutionary theory is a correct understanding of variation, both within and between living species and especially in relation to environment. It is also necessary to develop a theoretical model that describes the transmission and distribution of genetic information through time and space.

The evolutionary history of man and of the primates can now be partially reconstructed both through palaeontology and molecular anthropology but evolutionary history is not the whole story of evolution.

Furthermore the evolutionary interpretations of the human fossil record itself depends upon knowledge of principles of systematic zoology, of the morphological, behavioural and genetical variability in natural populations, and of the dynamics of evolutionary processes. Problems arise whenever human palaeontology has lost touch with central developments in evolutionary theory. The proliferation of scientific names for new fossil hominid discoveries is only one example of the effects of the past isolation of human palaeontology from other subjects.

As far as University College London is concerned, we would like to set straight the record. Two of our five teaching staff are predominantly concerned with teaching human and primate palaeontology and functional anatomy. Their status as 'temporary lecturers' does not reflect any low priority assigned to these subjects—it is the result of historical circumstances. We consider that these topics, as well as the study of human and non-human primate behaviour, make a contribution to the study of human evolution equivalent to that of biochemical and population genetics and other subjects. Accordingly, they occupy one half of the students' time during the first two years of the physical anthropology programme.

Dr Campbell's brief description of our research is a contracted version of an already condensed account in our departmental handbook. The latter had no space to mention that the study on the 'strength and structure of bone' is intended to clarify locomotor patterns in fossil man, or the palaeontological field-work carried out by two of our staff in East and South Africa and in Iran, most recently in 1976. We believe that our researches are relevant to the study of human evolution and that we would be no better placed in other departments.

We would like to add that professional human palaeontologists also work in London at the British Museum (Natural History) and at two London teaching hospitals. Altogether they number more than in any other city in the world. It is true that too many talented physical anthropologists have left for America. This is a regrettable occurrence, but it is not unique to physical anthropology, being primarily due to the effects of a harsh economic climate in Britain.

We conclude by assuring you that physical anthropology is alive and well, and living in Gower Street.

L. AIELLO

F. BRETT

D. A. COLEMAN

T. R. OLSON

H. M. WEYMES

University College, London

SIR,—We were dismayed to read Bernard Campbell's article on the state of physical anthropology in the UK. We consider it presents a totally false picture.

In the first half of this century there were two university teaching posts in physical anthropology in the country. There are at least 16 now, most of them in departments of physical/biological anthropology or anthropology. Further, many universities have established departments of human biology with large commitments to physical anthropology. The subject is also taught and/or researched in various departments of anatomy, in polytechnics and in the British Museum (Natural History).

The Society for the Study of Human Biology has a membership of about 450 of whom about 165 are resident in the United Kingdom. Most of these members teach or research anthropological genetics, growth and development, human ecology, environmental physiology, biological aspects of demography, primatology and palaeoanthropology—all central aspects of modern biological anthropology and all necessary for a proper understanding of human evolution. This hardly seems to us evidence of a dying subject!

We recognise that much of the research in fossil hominids is taking place in departments of anatomy and the British Museum. There are many good reasons for this, particularly the need for an extensive anatomical knowledge in palaeoanthropological research. We should point out, however, that wherever physical anthropology is taught in British universities, palaeoanthropology is recognised as an important component of the courses.

We agree that further expansion of physical anthropology is highly desirable; everywhere there is an increasing demand for it by both undergraduates and graduates. We support Dr Campbell in his view that further recognition of palaeoanthropology is necessary. We feel however, that he could only have written the article as he did through having been away from the dramatic developments in physical anthropology which have occurred in Britain in the last decade.

A. BILSBOROUGH

J. P. GARLICK

C. G. N. MASCIE-TAYLOR

PAMELA D. RASPE

*Department of Physical Anthropology,
University of Cambridge*

A. J. BOYCE

G. A. HARRISON

V. REYNOLDS

*Department of Biological Anthropology,
University of Oxford*

E. SUNDERLAND

*Department of Anthropology,
University of Durham*

news and views

H-2 restriction, the thymus and the immune response

from J. C. Howard

A RECENT issue of *Nature* carried a paper by Zinkernagel and colleagues (Zinkernagel *et al.*, 271, 251; 1978) which made a remarkable claim about the role of the thymus in the development of specific immune function by T lymphocytes. The claim concerns the origin of H-2 restriction in the specificity of T killer cells. The phenomenon of H-2 restriction was discovered in T killer cells developed in mice infected with certain viruses. Surprisingly, the killer cells would kill only virus-infected target cells sharing an H-2K or H-2D antigen with the infected host, but not cells infected with the same virus but sharing neither H-2K nor H-2D with the host. Essentially similar H-2 restriction has been found by others in the T cell mediated lysis of hapten-modified target cells and of targets differing from the immunised donor by minor (non-H-2) transplantation antigens. The concept of H-2 restriction has also been extended to cover the inductive interactions between macrophages, T helper cells and B cells which are apparently restricted in a similar way by the H-2I region.

A key question raised by the phenomenon of H-2 restriction concerns the range of specificity of the restriction. Is the specificity established exclusively during the induction of the antigen-reactive T cell, and hence defined simply as the H-2 specificity carried on the cell (macrophage?) membrane which presents antigen to the T cell, or is H-2 restriction limited only to the self H-2 specificity?

Until recently results have been in favour of the antigen presentation theory. For example an F_1 hybrid between two parents of different H-2 type can produce two independent sets of cytotoxic T cells, one restricted for each H-2 haplotype. The population which predominates can be controlled by presenting antigen in association with one or the other haplotype. Ex-

periments of this type have been used in support of the altered self, H-2 modification or interaction antigen theory of H-2 restriction, in which it is suggested that exogenous antigen forms a molecular association with H-2 in the presenting cell membrane. This theory explains the results of the heterozygote experiments since the two parental H-2 haplotypes, having distinct molecular structures, will generate two distinct interaction antigens in association with a single foreign molecule. The view that association with a single allotypic H-2 molecule might confer apparent allotypy on any other molecule in the membrane was expressed recently by Matzinger and Bevan (*Cell. Immunol.*, 29, 1; 1977) in general terms as a possible explanation for the great vigour of T cell responses to H-2 alloantigens. If H-2 was forming associations with all other membrane molecules, each such association would be recognised as a foreign antigen by T cell populations of a different H-2 type, resulting in massive and highly heterogeneous allogeneic responses. During immunisation with a conventional antigen the association of the foreign molecule with self H-2 would then constitute simply an antigenic example of a type of association forming constantly between H-2 and other self molecules in the membrane.

Although no detailed explanation in molecular terms has been forthcoming for the proposed ability of H-2 molecules to form close associations with antigens as various or as complex as minor transplantation antigens or viral antigens, the interaction antigen theory has until now had the advantage of simplicity, and of its ability to incorporate H-2 compatible and H-2 incompatible T cell reactions in a single scheme. It is implicit in such a generalised interaction antigen scheme for H-2 restriction that the heterogeneity or specificity range of the T cell receptor repertoire is very large, though presumably focused on H-2 specificities or their structural relatives. Thus the specificity of H-2 restriction

actually observed in a particular experiment would reflect the selective role of antigen seen by a T cell in association with the presenting H-2 specificity rather than a pre-existing limitation in the range of H-2 restriction imposed on T cells in some other way.

Zinkernagel and colleagues (*Nature*, *op. cit.*; *J. exp. Med.* (in the press)) and Bevan (*Nature*, 269, 417; 1977) have produced evidence which is difficult to reconcile with such a simple scheme in which the H-2 context of antigen presentation is the only condition determining the specificity of H-2 restriction. Both groups reconstituted irradiated parental strain (A) recipients with bone marrow from H-2 heterozygous (A $\times$ B) donors. After allowing time for the lymphomyeloid system to become repopulated with A $\times$ B cells, the recipients were immunised with minor (non-H-2) transplantation antigens (Bevan) or with vaccinia virus (Zinkernagel *et al.*). If the H-2 context of antigen presentation were the sole determinant of the specificity of H-2 restriction, and if the antigen-presenting cells (presumably macrophages) were of donor (A $\times$ B) origin, then cytotoxic T cells should develop that would lyse appropriate antigenic target cells of both A and B H-2 types. In the event, the cytotoxic response was largely (Bevan) or entirely (Zinkernagel *et al.*) restricted to the A H-2 type of the host.

This unexpected finding led both authors to suspect that the specificity of H-2 restriction might be some consequence of the environment in which T cells differentiate from their precursors. In a most important experiment, Zinkernagel *et al.* (*op. cit.*) showed that the relevant environment was the thymus, and thus could have nothing directly to do with the H-2 context of presentation of the immunising antigens, which occurs in peripheral lymphoid tissue. In this experiment, thymectomised A $\times$ B mice were irradiated, grafted with irradiated thymus tissue from either A or B

J. C. Howard is at the Institute for Animal Physiology, Babraham.

donors, and then repopulated with A × B marrow. The specificity of restriction of killer cells after virus infection was to the H-2 type of the thymus donor only.

It seems, therefore, that the precursors of T killer cells enter peripheral lymphoid tissue with a pre-existing bias towards restriction to the H-2 type of the thymus in which they differentiated. The H-2 context of antigen presentation can therefore select from only a limited repertoire of T cells. The thymus has been an intriguing organ for a long time, but this clear evidence that specificity of reaction of T cells is directly influenced by H-2 antigens carried on radio-resistant (presumably epithelial) cells of the thymus creates a whole new level of interest. It is remarkable that an effect of this kind was suggested on *a priori* grounds by Jerne as long ago as 1971 (*Eur. J. Immun.*, **1**, 1), and Jerne's speculations were invoked by Doherty *et al.* (*Transplant. Rev.*, **29**, 89; 1976) and by Bevan (*Nature*, *op. cit.*; *Cold Spring Harbor Symp. quant. Biol.*, **41**, 519; 1977) as a possible mechanism for the control of specificity of H-2 restriction before doing their critical experiments.

Every instance of H-2 restriction involves a cell interaction in which one of the partners is a T cell. This is as true for the interaction between the cytotoxic T cell and its target, restricted by H-2D or H-2K, as for the interaction between the T helper cell and the B lymphocyte, restricted by H-2I. Where the effector T cell, be it a killer or a helper, shows H-2 restriction, one can be confident that its precursor showed restriction of the same specificity when it was first induced by contact with antigen. This elementary proposition takes on a new significance now that the thymus is known to direct the specificity of H-2 restriction. In further experiments Zinkernagel *et al.* (*J. exp. Med. op. cit.*) have used the phenomenon of H-2 restriction to demonstrate that there must be a T helper cell involved in the generation of T killers, in much the same way that a T helper cell is involved in the stimulation of B cells. Furthermore, this T helper cell for the cytotoxic response is apparently restricted by the H-2I region.

They demonstrated this as follows. A rather special situation arises when an irradiated A × B recipient is reconstituted with A marrow. As the stem cells differentiate in the A × B thymus the T cells should acquire H-2 restricted reactivity to both A and B H-2 types. However, when they emerge into the periphery only the A type of antigen-presenting cell is available since the whole lymphomyeloid

Age of Midway revised

from Peter J. Smith

THE relevant geochemical, petrological and potassium-argon data are generally consistent with the view that the Hawaiian-Emperor island chain was formed as the Pacific plate moved across an underlying magma source fixed with respect to the mantle (although other explanations cannot yet be ruled out entirely). Certainly the volcanoes in the chain are of similar type; and they become progressively older away from the island of Hawaii, at least as far as Koko Seamount in the southern section of the Emperor limb. When the dating evidence is examined closely, however, certain irregularities become apparent; and some remain even when the data are adjusted to take account of known geological-geometrical relationships.

For example, Jackson and Shaw (*J. Geophys. Res.* **80**, 1861; 1975) noted that the Hawaiian chain volcanoes lie not exactly on a straight line but on slightly curved *en echelon* loci, which means that the measured distance of each volcano from the current hot spot position at Kilauea (Hawaii) is probably not the true geological distance. So Jackson *et al.* (*Earth planet. Sci. Lett.* **26**, 145; 1975), assuming volcanoes on parallel loci to be synchronous, recalculated the volcanic distances from Kilauea and showed that the new 'collapsed distances' give a more uniform age progression along the chain. But not all irregularities disappeared even then, the most conspicuous of those remaining being the age of Midway island. According to Jackson *et al.* this should be 24.6 Myr although Dalrymple *et al.* (*Bull. Geol. Soc. Am.* **85**, 727; 1974) obtained a K-Ar age of 17.9 ± 0.6 Myr.

It is important to get this age right, for as Dalrymple *et al.* (*Earth planet. Sci. Lett.* **37**, 107; 1977) now point out, the position of Midway is such that its age has a disproportionate influence on such matters as the determination by extrapolation of the age of the Hawaiian-Emperor bend, tests for the stability (or otherwise) of hot spots, and the calculation of rates of volcanic propagation. Accordingly, Dalrymple and his colleagues have redetermined the age of Midway using alkalic basalt pebbles from the conglomerate which overlies the tholeiitic flows used in the previous dating study. They have also carried out $^{40}\text{Ar}/^{39}\text{Ar}$ incremental-heating experiments which indicate that the tholeiites are unsuitable for reliable K-Ar dating and thus that the original age of 17.9 Myr must now be regarded only as a minimum.

The new K-Ar age (from the pebbles) is 27.0 ± 0.6 Myr. This is rather greater than the age predicted by Jackson *et al.*, which suggests that not all the lack of uniformity is due to incorrect dating. On the other hand, the new age removes the need to assume large changes either in the rate of rotation of the Pacific plate about the Hawaiian pole or in the motion of the plate relative to the Hawaiian hot spot since the formation of the Hawaiian-Emperor bend about 42 Myr ago. Thus all the age data from the Hawaiian chain are now reasonably consistent with a uniform rate of volcanic propagation of 8.0 cm yr^{-1} or an angular rotation of 0.83° per Myr about a pole at 69°N , 68°W .

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

complex including macrophages is repopulated with donor (A) type cells and hence only the A-restricted population of T killer cells is activated by virus infection. The existence of the B-restricted population can, however, be demonstrated if lymphoid cells from the A → A × B chimaera are transferred to recently irradiated virus-infected recipients of A × B type. In these recipients the antigen-presenting structures are still of host (A × B) origin, and both A and B restricted populations of killer cells develop from the donor inoculum. The important concept here is that in the appropriate experimental circumstances T cells can apparently become restricted in the thymus to H-2 specificities which they do not themselves carry, and with which antigen is never presented in the peripheral

lymphoid system in which they operate. The extreme case of this 'diversionary' restriction is in the fully allogeneic chimaera A → B, when all T cells become restricted to an H-2 type that neither they nor any other lymphomyeloid cell of that individual carries. In such a chimaera, therefore, no H-2 restricted T cell responses should develop at all: all T cells are B-restricted while all antigen is presented with A. It has in fact been known for some time that such allogeneic chimaeras show an extraordinary spectrum of immunoincompetence in responses now known to be H-2 restricted, and Zinkernagel and his colleagues have confirmed this yet again in the virus-specific cytotoxicity system.

But now consider what would be expected if lymphoid cells from an

A $\rightarrow$ B allogeneic chimaera, itself unresponsive to virus infection, are transferred to a virus-infected recently irradiated A $\times$ B recipient. Since the irradiated recipient presents virus with B H-2 type one would at first expect B-restricted virus-specific cytotoxicity to develop. But it does not. Zinkernagel *et al.* explain this superficially unexpected failure most beautifully. They argue that the generation of T killer cells from their precursors requires antigen-specific T help, and that this help, like antigen-specific T help for B lymphocyte induction in antibody formation, is itself H-2 restricted. It is known that the H-2 restriction requirement of a T helper cell can only be satisfied if the B lymphocyte itself carries the restricting H-2 type. If the same condition were to apply to T help for the generation of T killer cells, then T helper cells from the A $\rightarrow$ B chimaera would be unable to provide help for T killer cell precursors from the same chimaera, even when stimulated by virus in a second irradiated A $\times$ B host. Since the T killer cell precursors are of A strain origin, they cannot be helped by activated T helper cells restricted to B. In a long and complex series of experiments Zinkernagel *et al.* go a long way towards demonstrating the validity of

their hypothesis, and show that the provision of T help for killer cell precursors is probably restricted by H-2I, just as is T help for B lymphocytes in antibody production.

It is difficult to overstate the importance of these experiments. They demonstrate conclusively that the thymus has a selective role in the development of T cell specificity based on the recognition of self H-2 structures, a finding that will generate a whole area of theory and research. At the same time they provide the strongest challenge so far to the simple interaction antigen theory of H-2 restriction, and, as the authors point out, their results strongly favour the proposition that T cells may carry two functionally independent receptors, one for 'self' H-2 defined by the H-2 type of the thymus, and one for exogenous antigen. Finally the demonstration that the differentiation of T killer cell precursors to killer cells requires an H-2I restricted interaction with an antigen-specific T helper cell creates an elegantly symmetrical model for the T helper cell mediated induction of the two specific effector modes of the immune response, T killer function and antibody formation. $\square$

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Fig. 1 The human cervical vertebrae. Total descriptive accuracy.

he wanted, and he suggests in his writings that the new and precise technique of copperplate engraving would be appropriate. But the task was too much for his literary heirs, and the drawings remained a legend, locked away and seen only by a chosen few, until William Hunter saw them at Windsor in the late eighteenth century.

It is not hard to see why this happened. By no means all the drawings are anatomical masterpieces. Apart from those of his last phase, done at Florence when he lived in the hospital of Santa Maria Nuova and was able to see and take part in the medical school dissections, and also was associated for a short time with a professional anatomist, Marcantonio della Torre, they fall into perhaps three categories. The first, and to an anatomist the most important, are those where he drew what he actually saw (Fig. 1). The second are where he has transposed observations on animals to the human body (Fig. 2), distorting outlines where necessary to fit the outlines of the human figure, or is relying on memory; and the third is where he is interpreting anatomy in terms of the dogmas of the day—the teachings of Galen, Avicenna, Mundinus and others. This led to some weird interpretations, such as that in the picture of coition between a hemi-sectioned man and woman (Fig. 3), where

An anatomist's look at Leonardo

from E. H. R. Ford

THE exhibition at the Royal Academy in London of Leonardo da Vinci's anatomical drawings (from the Royal collection at Windsor) has been the first opportunity for the public in this country to see the actual drawings (as opposed to photographs). Leonardo has aptly been described as the man who awoke too early in the darkness, while all the others were still asleep.

Although it is likely that the anatomists of the time knew more than we give them credit for, his drawings contain the first description we have of so many things; the

maxillary outrun, eponymously credited to Highmore, 1651); the meningeal arteries; the first accurate representation of bones (his drawings of the vertebral column, and especially the cervical vertebrae, are as good as those in any modern textbook); the first use of cross-sectional anatomy; the first realistic picture of the foetus *in utero*, of the ventricles of the brain by injection, the list is long. And yet all this work had a minimal impact on his contemporaries, because it was never published at the time. There is little doubt that publication is what

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Fig. 2 The nerves and bones of the arm. Extrapolation from animal dissection; drawn as human, but the humerus obviously is not.

to have been left-handed), and ran words together often unpredictably (perhaps to prevent the inquisitive stealing his ideas). There was little proper anatomical vocabulary at that time, so it was hard for him to describe much of what he drew in terms which others could understand. It is not perhaps surprising therefore that Francesco Melzi, his favourite pupil and literary heir, was unable to put together a fifteenth century Gray's

Anatomy from the mixture of fantastic artistry, accurate observation, extrapolation from animal studies and mediaeval misconceptions. After Melzi's death the material passed to the sculptor Pompeo Leone who took it to Spain, and when he in turn died it was dispersed, and after a period reached the Royal Collection, probably in the time of Charles II. Since then it has been published and described several times. □

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Crops for the desert

from Peter D. Moore

ONE of the major problems of irrigation agriculture in the semi-arid parts of the world is salinity. Both in coastal areas and in many inland salt-desert regions, the water available for irrigation is high in dissolved salts, which makes it unsuitable for most crop plants. Desalination is expensive, though it may be considered worthwhile among the energy-rich nations; but the alternative, to seek varieties of crop plants which are salt tolerant, has only recently been attempted.

Work in Australia by Morgan (*Nature* 270, 234; 1977) has shown that wheat genotypes vary considerably in their capacity to osmoregulate under different conditions of soil water stress. This capacity to adjust internal osmotic potential in response to environmental stress could

be advantageous under saline conditions as well as providing drought hardness. Evidently there is room for selection within the wheats.

Some direct, experimental demonstrations of the potential of the cereals has been reported by Epstein and Norlyn (*Science* 197, 249; 1977). In California they have examined the capacity of several cultivars of barley to cope with saline irrigation. Using one variety (Composite Cross XXI, Fq) they began salinisation when the seedlings were 8 d old and gradually increased the NaCl concentration of the medium until it reached 75% that of seawater. At the end of the experiment 5.9% of the plants had managed to complete their life cycle. A similar experiment was run using a marine mix instead of NaCl and this time 9.2% completed

Fig. 3 Coition between a hemi-sected man and woman, demonstrating mediaevel concepts of anatomy and physiology. The penis has two passages—the upper receives semen from the spinal cord.

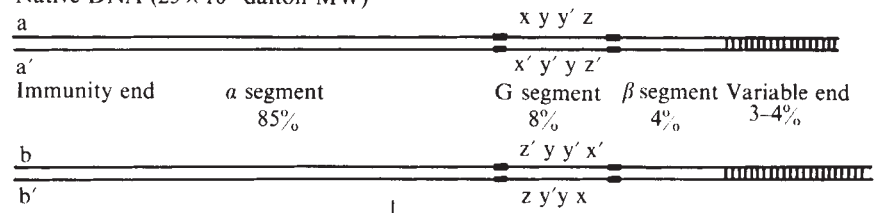
the penis has a double channel; the upper carries semen from the lumbar spinal cord, the lower semen from the testes and urine from the bladder.

Most of the more unrealistic drawings date from Leonardo's early period, before he had much experience of dissection. It is not at all certain how much experience of dissection he actually had; he claimed to have dissected over 30 bodies, but some of these dissections may have been fairly slight. O'Malley-Saunders (*Vesalius on the human body*; New York 1952) considers that the hard evidence points to only a few detailed dissections; a human head and neck in his early period; a centenarian and a child of two at Florence; a foetus of about 7 months; a middle-aged man and perhaps a younger man; and possibly a separate leg. He probably did not have a proper skeleton, although he had some parts, including a skull.

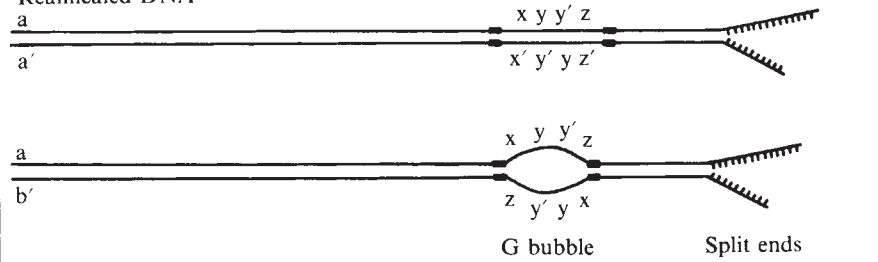
In addition to the mixture of fact and extrapolation in some of the drawings, Leonardo's written descriptions must have been hard to follow. He used mirror writing (he is thought

Erratum

Native DNA (25×10^6 dalton MW)



Reannealed DNA



The second and third DNA diagrams in Fig. 1 of the article 'Invertible DNA in phage Mu' (*News and Views* 271, 608; 1978) were wrongly lettered. The correct figure is reprinted above.

their life cycle. Seed from these two experiments was then increased ready for a further experiment in which an 85% seawater mix was supplied immediately after planting. Later in the experiment the salinity was reduced, but only 0.31% survived.

This rigorous selection process was followed by further seed multiplication and by field trials using three levels of salinity, 100%, 67% and 33% seawater. Very little rain fell during the course of the experiment, so irrigation was by far the major source of water. Germination, growth, flowering and seed production took place under all conditions. The feed quality of the grain was satisfactory and the yields of grain produced varied from 5.6 to 75.8 g m⁻². Extrapolation from such figures would put yield expectations at around 800 kg ha⁻¹ with the better genotypes reaching over 1,000 kg ha⁻¹.

Peter D. Moore is a Senior Lecturer in the Department of Plant Sciences, King's College London.

This figure is more than half the mean world yield for barley in 1975 (1,710 kg ha⁻¹), which is not inconsiderable when one takes into account the salinity under which it was achieved.

This is a first step in what will undoubtedly prove to be a protracted process of genetic sieving within the barley varieties, but it clearly demonstrates the capacity of this cultivated species, in both its phenotype and genotype, to cope with salinity.

Epstein (In *Plant Adaptation to Mineral Stress in Problem Soils*, (ed. Wright, M. J.) Cornell University, 73; 1977) has also begun a similar project with wheat, but no field trials have yet been conducted. Initial results suggest that saline-tolerant genotypes are present and can be selected for. There seem to be considerable opportunities for improving existing crops by simple selection apart from the as yet unexplored possibilities of cross-breeding with closely related wild genotypes, many of which still survive in the semi-arid areas of the Middle East where cereal cultivation first began. □

Clinical trial methodology

from Richard Peto

DURING the past quarter of a century, more and more questions about the treatment appropriate for particular categories of patient have been settled by large randomised clinical trials. Patients are allocated completely at random to one of two or more alternative treatments, as has been done in agriculture since much earlier in the century, to discover which is the most effective of the treatments being compared. However, in medical science it is extremely difficult to study large enough numbers of patients to distinguish reliably between a moderate difference and no difference, and for uncommon diseases this may only be possible by organising collaboration between many hospitals. (Diseases so rare that even this fails are not considered here). Moreover, if the disease is potentially fatal then any system of experimentation involving a concurrent control group means that one or other group of patients may be denied possibly life saving therapy.

In practice, medical investigators frequently avoid the scientific rigour of the large randomised trial, either by organising a randomised study which is so small that it could only reliably detect an implausibly gross improvement in disease outcome, or by avoiding randomisation and treating a whole

series of patients with one particular treatment, comparing the observed results with 'historical experience'. These practices cannot be wholly bad, since they have been the basis of most advances in therapy.

However, moderate therapeutic improvements in common diseases may be of more value than substantial improvements in rare ones. So it is important to recognise moderate therapeutic improvements and also to discover which of today's expensive, toxic or inconvenient treatments confer no material benefit on the patient. For these purposes, studies comparing two treatments must distinguish between a moderate difference and no difference and it is especially here that the large randomised trial is advisable. It has often been argued (and demonstrated) that whatever precautions are taken to exclude biases in small or non-randomised studies, random or systematic errors may well remain, producing effects in either direction much greater than the effects of moderate treatment differences. This is illustrated yet again by a recent review by Chalmers *et al.* (*New Engl. J. Med.* 297, 1091; 1977) of the remarkable history of the failure over 25 years to evaluate properly anti-coagulant treatment for heart patients who have been admitted to hospital immediately following a sudden myocardial infarction, the commonest cause of death in

developed countries.

It is clear that either inhibitors of coagulation or anti-platelet agents might be of therapeutic value (either preventing venous or arterial thrombosis) following myocardial infarction. But it is also clear that gross interference with an essential physiological function such as haemostasis carries some inherent risks of cerebral or other haemorrhage and it is not intuitively obvious which categories of patients if any, will benefit from anti-coagulant therapy. Between 1948 and 1975, 32 papers (involving a total of 3,400 deaths among 16,000 myocardial infarction patients) have been published describing studies of anti-coagulant therapy. These include 18 historically controlled, non-randomised studies (containing an average of 500 patients per study), 8 concurrently controlled non-randomised studies (average 400/study), 3 small randomised studies (average 100/study) and only 3 large randomised studies (average 1,200/study, published 1969-73).

In the pooled randomised studies 14.3% of the treated patients died as against 17.9% of the controls (a 20% reduction in the risk of death, with approximate 95% limits of 5%-35% reduction) and in the concurrent non-randomised studies there was an apparent 27% reduction. By contrast, in the historically controlled studies 14.9% of the treated patients died as against 31.6% of the supposed 'controls' (a 53% reduction, with approximate 95% limits of 46%-60% reduction).

Why is the ratio of the proportion of treated patients dying to the proportion of controls dying so much more promising in the 18 historically controlled studies (53% fewer deaths) than in the concurrently controlled studies (20% or 27% fewer deaths?). This discrepancy is far too great to be due to chance, and so only three possibilities remain. Perhaps the organisers of the historically controlled studies got patients who were more likely to need anticoagulation (although all studies had fairly unrestricted intake), perhaps they gave better anticoagulation (although the overall results among the treated patients were no better) or perhaps, as Chalmers *et al.* conclude, inappropriate historical controls were selected. If so, then although 'historical comparisons' are still a very common way of comparing treatments, the biases inherent in this common methodology are of the same order of magnitude as the effects which one commonly wishes to estimate. Thus, even if anticoagulation were without any material benefit, statistically significant results would probably have emerged from many of the non-randomised historically controlled

Richard Peto is Reader in Cancer Studies at the University of Oxford.

studies. Suspicion that this might be so is perhaps one cause of the general reluctance over the past 30 years to use anticoagulants routinely, during which period literally millions of patients have presented after a sudden myocardial infarction in hospitals around the developed world. It is not clear exactly how many of these would have been saved had convincingly objective large randomised trials been undertaken in the 1940s and 50s to determine which categories of patients should, and which should not, be anticoagulated, but the number could be large. (Since the chance variation that could occur in the small randomised studies considerably exceeded the effects of a 20% reduction in mortality, none of them yielded statistically significant results).

Fortunately, when anti-platelet agents were introduced in the 1970s for patients who have recovered from a myocardial infarction, really large randomised trials of various anti-platelet agents were undertaken (the most recently reported being the Anturane reinfarction trial (*New Engl. J. Med.* **298**, 289; 1978)) and clear answers should be available by 1979. Also, large randomised trials of the primary prevention of myocardial infarction and stroke by prophylactic anti-platelet agents in apparently healthy people are now being attempted. When, as is often the case, the differences between alternative medical treatments are unlikely to be gross, strict scientific rigour appears to be advisable in clinical research.

□

Freeze-fracture view of membrane fusion

from David Shotton

FREEZE-fracture electron microscopy is unique in enabling us to look directly at the two-dimensional distribution of integral proteins within biological membranes. Several laboratories have recently applied this technique with considerable success to the study of membrane fusion, and the past year has seen a flurry of papers reporting their results.

Membrane fusion is one of the most ubiquitous cellular events. It is most easily studied, however, in cells which secrete proteins or other synthetic export products by exocytosis from large membrane-bound secretory vesicles or storage granules.

The most favoured system has been the degranulating peritoneal mast cell. Chi *et al.*'s initial report (*Proc. natn. Acad. Sci. U.S.A.* **73**, 2823; 1976) has been supported by those of Lawson *et al.* (*J. Cell Biol.*, **72**, 242; 1977) and of Burwen and Satir (*J. Cell Biol.*, **73**, 660; 1977), in which the freeze-etch results are more fully complemented by labelling and thin-section studies. Very similar studies have also been published on mucus-secreting goblet cells by Neutra and Schaeffer (*J. Cell Biol.*, **74**, 983; 1977) and most recently on pancreatic B cells by Orci *et al.* (*J. Cell Biol.*, **75**, 23; 1977). In each case the authors have interpreted the various static electron micrograph images obtained from their freeze-fracture replicas in terms of a series of steps in the dynamic processes of membrane fusion.

Initially the large secretory vesicles come close to the plasma membrane,

which bulges out as it adopts the curvature of the underlying vesicles. Most significantly, these bulging areas of close membrane contact are totally or almost completely devoid of intramembrane particles. The particles themselves, formed of large integral membrane proteins, are found instead at somewhat increased densities around the margins of individual bulges or crowded together in the limited areas of 'normal' membrane left between adjacent bulges.

Lawson *et al.*, following an earlier suggestion of Farquhar and Palade (*J. Cell Biol.*, **17**, 395; 1963), make a useful distinction between membrane 'fusion' and the subsequent membrane 'fission' or rupture. Once the membranes have become closely apposed, fusion is usually marked in thin section by a change from the normally trilaminar appearance of the two apposed membranes to a single pentalaminar structure ~125 to 135 Å thick. In freeze-fracture studies this stage cannot be distinguished from the unfused state immediately preceding it, and so the basic bilayer arrangement of the membrane phospholipids from each membrane probably remains more or less intact. At fission the fused membranes rupture, releasing the vesicle contents to the extracellular space. Although calcium ions have been widely implicated in the initiation of membrane fusion, the trigger for this rupture and the molecular rearrangements involved are not yet known.

Lawson *et al.* have shown by labelling experiments that the areas of close approach and fusion between vesicle and plasma membranes are devoid not

only of intramembrane particles but also of the normal complement of membrane-bound immunoglobulins and of receptors for the lectins Con A and PHA. Thus a necessary precondition for membrane fusion in these cells seems to be the removal of most, if not all, of the membrane protein from both participating lipid bilayers.

This idea has gained further support from freeze-fracture studies of the fusion of peripheral vesicles with the plasmalemma of zoospores of the fungus *Phytophthora palmivora* during their encystment (Pinto da Silva & Nogueira, *J. Cell Biol.* **73**, 161; 1977) and of the fusion of acrosomal and plasma membranes during the acrosomal reaction in guinea pig sperm (Friend *et al.*, *J. Cell Biol.*, **74**, 561; 1977) in which the sites of membrane fusion are also particle-free.

The idea that membrane fusion is primarily lipid-mediated, requiring the initial depletion of membrane proteins, was originally suggested in 1975 from quite separate and indirect evidence (Ahkong *et al.*, *Nature*, **253**, 194; 1975, building on earlier proposals by Lucy, *Nature* **227**, 815; 1970).

The apparent agreement of the results described here disguises a fundamental difference in interpreta-

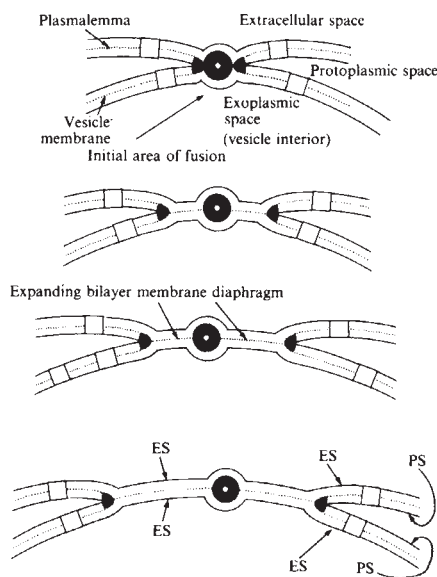


Fig. 1 The diagram represents steps during membrane fusion leading to the formation of a bilayer membrane diaphragm. The dotted line follows the hydrophobic junction which is split on freeze-fracture. Solid black areas represent the postulated central inverted membrane micelle (circles) and expanding toroidal hemimicelle (wedges). Lipids may flow from the external half of the plasma membrane and the inner (exoplasmic) half of the vesicle membrane into the bilayer diaphragm, but integral membrane proteins spanning the plasma and the vesicle membranes (squares) are unable to enter this area. (ES, extracellular or exoplasmic surface; PS, protoplasmic surface). From Pinto da Silva & Nogueira.

David Shotton is a lecturer in the Department of Biochemistry at Imperial College, London.

tion of the fusion events between some of the authors. Although Chi *et al.* reported relatively rare cases in which the fusion bulges appeared in thin section as a central dense line with very fine striations forming a fuzzy coat on either side of the line, both they and most of the other authors seem to regard the pentalaminar appearance of the fused membranes as the norm, being the thin section image of two intact bilayers in close apposition. In contrast, Pinto da Silva and Nogueira report the fused membranes formed between peripheral vesicles and the plasma membrane of *Phytophthora palmivora* to be trilaminar particle-free single bilayer structures, which they term membrane diaphragms. They suggest that these are formed of lipid derived from the exoplasmic and extracellular (E) halves, respectively, of the two fusing membranes, the two protoplasmic (P) halves having initially undergone a local catastrophic molecular rearrangement to form an expanding toroidal hemimicelle (nomenclature of Branton *et al.*, *Science*, **190**, 54; 1975). This both provides continuity between the two P half-membranes adjacent to the area of fusion and also effectively excludes intramembrane particles (integral proteins spanning the entire bilayer) from entering the growing particle-free bilayer membrane diaphragm formed from the E half-membranes (Fig. 1, reproduced from Pinto da Silva & Nogueira). In simplistic terms, their model resembles the rearrangements which occur when two soap bubbles meet and fuse. While their membrane diaphragms appear indisputably trilaminar in thin section, the peculiar geometry of the fusion process in the zoospore they have chosen to investigate leads to complex freeze-fracture images whose interpretations are not self-evident. However, the data presented do not easily accommodate the conventional interpretation, and the absence of the smooth particle-free prefusion membrane bulges characteristic of the mast cells, pancreatic B cells and goblet cells investigated by the other workers suggests that fungal and mammalian membranes may indeed behave differently during fusion. What is heretical about the suggestions of Pinto da Silva and Nogueira is the idea that a membrane can be composed of two E half-membranes, rather than an E half and a P half, although this topological infringement of the membrane biologists' central dogma is only transitory, to vanish as soon as the membrane diaphragm ruptures to release the vesicle contents. Despite the fact that their suggestions are not clearly supported by freeze fracture or thin section data from any other system, they

merit consideration because they bring the debate into the molecular realm with a thermodynamically reasonable model which explains the observed particle exclusion.

The most crucial questions which remain unanswered are those of the specificity and the mechanism of membrane fusion events. Virtually nothing is known about the involvement of membrane-bound cytoskeletal elements in bringing fusing membranes into close apposition, about the receptors and recognition proteins which specify and regulate both intracellular and exocytotic fusion events, and about the details of the catalytic processes and molecular rearrangements involved. □

High- β tokamaks?

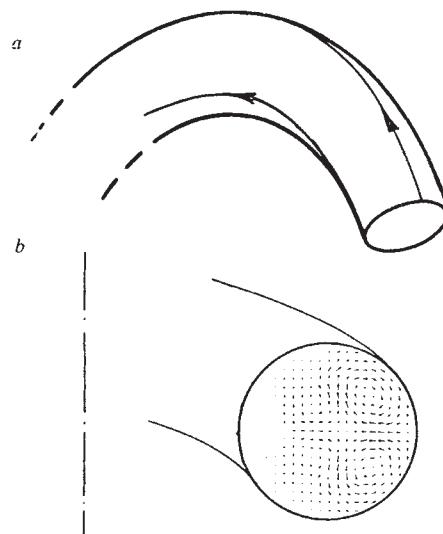
from John Wesson

THE nuclear fusion research programme is currently undergoing a considerable change. Over the past few years there has been an increasing concentration on the toroidal magnetic containment systems called tokamaks. It has become apparent that larger experiments are required and the proposed JET (Joint European Torus) tokamak, and a similar device (TFTR) in the United States represent a considerable increase in scale over present experiments. In parallel with this development there has been a determined effort to discover the theoretical limitations on the conditions which will be achievable in a tokamak fusion reactor. This effort has recently been concentrated on the question of the amount of plasma pressure which can be confined by the practically available magnetic fields. An indication of this interest is the publication, during the last year, of six contributions on the subject in *Physical Review Letters*. These papers are all concerned with a quantity— β —which is a measure of the plasma pressure.

First, what is β and why is it important. To obtain a sufficient burn of the deuterium-tritium fuel a temperature (T) around 10^8 K is required. At a given temperature the rate of nuclear reactions is proportional to the square of the plasma density (n). Thus the power per unit volume, which is a rough measure of power per unit cost, increases as the plasma density increases. For an economic reactor a sufficiently large value of n must be achieved.

The plasma pressure p is proportional to the product nT and we see

John Wesson is at the Culham Laboratory (Euratom/UKAEA Fusion Association).



a, In tokamaks, the magnetic field curvature on the inside of the torus is away from the plasma and stabilising. On the outside it is towards the plasma and destabilising. At low β the stabilising and destabilising effects are averaged and the plasma displacements are almost symmetric. It is then comparatively easy to achieve stability. *b*, At higher β the plasma displacements are concentrated in the unstable region. The stabilising effect of the good curvature is then much less and the configuration can become unstable.

therefore that, since T is essentially determined, the requirement of sufficiently high n implies a sufficiently high pressure. Now if the pressure is increased beyond a certain value in a given magnetic field the plasma becomes unstable. With a higher magnetic field, it is possible to achieve a higher pressure but unfortunately, with present technology, the average magnetic field in a reactor will probably be limited to a value around 40 kilogauss.

It turns out that the limit to the stable pressure is a given fraction of the magnetic energy density. It is convenient therefore to discuss the problem in terms of the ratio of the pressure to the magnetic energy density and this is in fact the quantity β introduced above. Since the magnetic energy density is $B^2/8\pi$ we have

$$\beta = \frac{p}{B^2/8\pi}$$

We see then that on the one hand the value of β must be sufficiently high for a reactor to be economic, and on the other that stability places a limitation on the value of β which can be obtained. Calculation of the β required for economic operation depends somewhat on the assumptions made but current estimates are around 10%.

In present experiments β is usually less than 1%. Theoretical analysis shows that plasmas of this type are stable to the grossest modes of in-

stability. These are called magneto-hydrodynamic (mhd) instabilities and are similar to the instabilities of conventional fluids. The present intense interest arises from the challenge of finding whether this low β value can be raised by the required order of magnitude. It is believed that the necessary energy can be provided by the injection of powerful beams of particles and by the nuclear reactions themselves but the question of stability at the higher β values must be resolved.

In computer calculations by Wesson and Sykes (*Proc. 5th Int. Conf. Plasma Physics and Controlled Nuclear Fusion Research*, Tokyo, Vol. 1, 449; 1974) we found that instabilities tended to be stronger on the side of the plasma away from major axis of the torus. The reason for this is that the curvature of the magnetic field is destabilising in this region whereas it is stabilising on the side of the plasma nearest to the major axis. However, at sufficiently low β , instabilities see an average of these curvatures and it can easily be arranged that this average is such as to produce stability.

Two letters published in April 1977 (*Phys. Rev. Lett.*, **38**; 1977); Todd *et al.*, 826; Bateman & Peng, 829) gave the results of further computer calculations carried out in the United States, for higher β . It was found that as the β value is increased the instabilities become increasingly localised in the unfavourable region and the averaging is less effective. The change in the type of mode as β is increased is illustrated in the figure. The arrows indicate the motion of the plasma. The first of the two papers mentioned found a configuration with $\beta = 2\%$ which was stable to large scale perturbations. The other gave results for configurations with β up to 5%. In this case the plasma boundary was regarded as fixed and a configuration stable to large scale perturbations was found with $\beta \approx 5\%$. It is not known whether it would be stable with the more realistic condition of a free boundary.

In September, calculations carried out at the Culham Laboratory (Sykes *et al. Phys. Rev. Lett.*, **39**, 757; 1977) demonstrated stability, again for large scale modes and a fixed boundary, for a partially optimised configuration with $\beta = 12\%$. This result was encouraging since it took the β values into the range needed for a reactor. However, it remained necessary to explore the importance of small scale instabilities which the numerical calculations described above cannot resolve and also of the effect of removing the constraint of the mixed boundary.

From these computer calculations it became apparent that, within the plasma, the small scale instabilities are the most difficult modes to stabilise. A

comparatively simple method of describing these instabilities was found by several workers in the United States (Coppi, *Phys. Rev. Lett.*, **39**, 939; 1977; Dobrott *et al.*, 943) and in Britain (Connor, *et al. Phys. Rev. Lett.*, **40**, 396; 1978). Using computers this method has been applied to several possible plasma configurations. The initial values of β which were found to be stable were quite low, around 1 or 2% but values up to 10% have since been obtained.

However, this is not the final answer since these calculations do not include those instabilities which only arise when the free nature of the plasma boundary is taken into account. To some extent there is a conflict between the stabilisation of the small scale modes of instability described above and the free boundary modes. This can only be resolved by numerical computations and many groups throughout the world are now working intensively toward the complete optimisation of β in order to determine whether the optimum is sufficiently high to make a fusion reactor an economic possibility.

Rescue archaeology: new approaches needed

from Jane Renfrew

CONCERN over the inadequate financial provision for rescue archaeology in the United Kingdom emerged as the overriding preoccupation of a recent conference organised by students in the Department of Archaeology at the University of Southampton.

Although the government has given considerable support to rescue archaeology in recent years, this still does not meet demand. This lack of resources exacerbates the considerable problem of selecting which sites of a number threatened should be excavated. It also leads to inadequate work, often because people have to move to other jobs before their work is completed. The lack of any secure career structure within rescue archaeology, especially for field archaeologists, means that it is often difficult to maintain the high standards which these jobs demand.

Another matter for concern is the lack of research design in much rescue archaeology. It was widely felt that there was too great a preoccupation with collecting more and more data in rescue excavations rather than asking and seeking answers to funda-

mental questions about past societies and how they worked; theories should be tested in the field, and the licence to think about problems of the past should not be restricted to academics. Much more free and open discussion of the theoretical background to archaeological interpretations should be encouraged.

Of the sites imminently threatened, it was felt that those in the ancient English cities where development was taking place were more or less adequately covered by the urban rescue units set up by the Department of the Environment. However, the devastation of ancient sites in rural areas by deep ploughing needed urgent attention, quite apart from the problems posed by road building, laying pipelines and gravel quarrying, which are more limited in area and effect.

The conference was held soon after the Ordnance Survey had made clear their intention of running down their Archaeology Division. This division has hitherto maintained a national record of archaeological sites in Britain and the conference stressed the importance of maintaining this record not only as a comprehensive record of a consistently high standard to which research workers can turn, but also as an invaluable aid to rescue archaeologists in assessing the amount of damage likely to follow from development. The conference has asked the Secretary of State for the Environment to take note that 'the archaeological resources of the Ordnance Survey for fieldwork, for recording and for cartography are of crucial importance for British archaeology both in terms of the experience and skill of the staff of the Archaeological Division and in terms of financial resources; that those resources should not be lost to British archaeology; and that we ask the Secretary of State for the Environment to take note of the unanimous view of this conference'.

However, comfort can be drawn from some of the successes of archaeology within Britain over the past century including The Ancient Monuments Act of 1882 which has given real protection to sites either by taking them into guardianship or by scheduling them. British archaeology has laid the foundations for systematic fieldwork, excavation and archaeological interpretation in Europe and must not be allowed to lose this tradition. There is also a great interest in archaeology on the part of the general public and a long tradition of informed amateur field archaeology. The conference felt that there was even greater room for public involvement in local archaeological work, and that the public, as tax-payers contributing to the financing of many excavations, should be kept more fully informed of the results. □

Jane Renfrew is a visiting lecturer in the Department of Archaeology at the University of Southampton.

articles

Cl and Br degassing by volcanism along the Reykjanes Ridge and Iceland

C. K. Unni & J-G. Schilling

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881

Chlorine and bromine variations in basalts erupted along the Reykjanes Ridge axis and Iceland follow a predicted first-order model, reflecting volatile degassing at water depths shallower than about 500 m, superimposed with changes due to the presence of two chemically distinct mantle sources and their mixing. Iceland mantle 'plume' contains three to four times more pristine Cl and Br than the asthenosphere depleted in large ionic lithophile elements.

THE origin of water, Cl, CO₂ and other volatiles accumulated as oceans and atmosphere is generally believed to be due to the progressive degassing of the Earth's interior by volcanism throughout geological time^{1,3}. Verification of such a hypothesis by direct measurements of input rates of gases at volcanic centres has been complicated by the danger and difficulties involved in sampling the problem of contamination by atmospheric gases or the incorporation of heated subterranean fluids, and differential condensation. An independent approach was pioneered by Moore^{4,5} who looked at volatile contents of submarine basalts erupted and quenched under varying hydrostatic pressure (or depth of water) along the submerged flank of Kilauea Volcano, Hawaii. He found that the H₂O content of basalt dropped rapidly above 500 m depth. This critical depth of water corresponds to the hydrostatic pressure at which basaltic magma at 1,000–1,200 °C reaches saturation, as theoretically predicted by McBirney⁶. Subsequent studies⁷ showed that sulphur also drastically degasses at the same depth as water, despite that sulphur solubility would seem to have been exceeded at higher pressure. Results for Hawaii were further confirmed from basalts erupted along the Reykjanes Ridge and extension over Iceland by Moore and Schilling⁸. These authors suggested that H₂O during its effervescence from magmas may act as a fluxing agent for other volatiles. Such a mechanism remained to be tested for other volatiles such as Cl, Br and F. For this purpose, and that of estimating volcanic input rates of volatiles and the Cl and Br content of mantle sources of basalts from mid-ocean ridges and unusual hotspot regions such as beneath Iceland, we undertook a detailed study of submarine and subaerial basalts from the Reykjanes Ridge and Iceland (Fig. 1).

Model prediction for testing and results

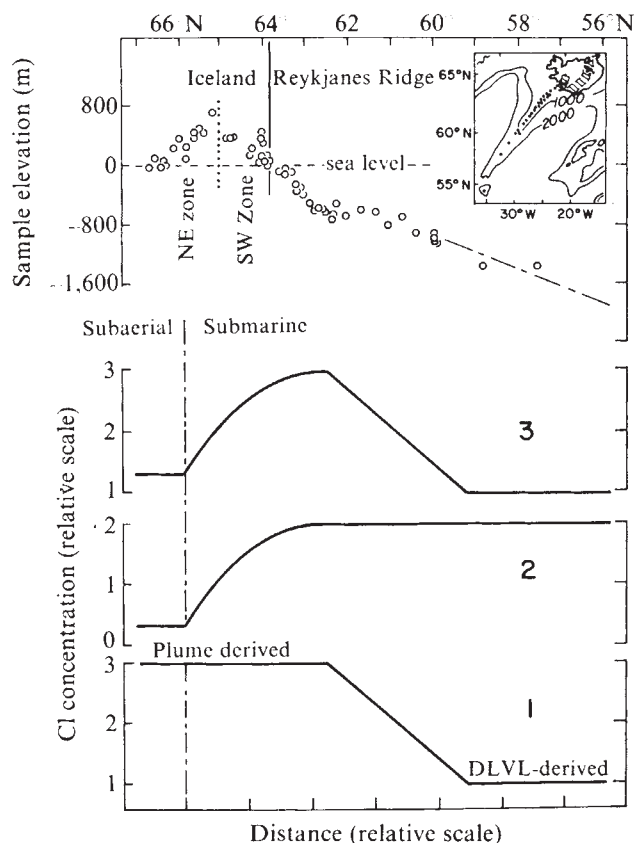
Water depth increases regularly from Iceland southward along the Reykjanes Ridge axis, thus offering an ideal natural laboratory for studying the effect of pressure on degassing of volatiles, and mechanism of degassing by volcanism in general (Fig. 1).

Combining the empirical data on H₂O and S in basalts derived from a single mantle source along the flank of the island of Hawaii^{4,5,7} and concentration gradients of large ionic lithophile elements (LILE) and radiogenic Sr and Pb isotopes along the

Reykjanes Ridge which reflect two mantle sources and their mixing^{9–11}, we have constructed for testing, a first-order model describing the geographical distribution of the large volatile anions Cl and Br along the Reykjanes Ridge–Iceland profile (Fig. 1).

Curve 1 sketches the hypothetical concentration profile of Cl or Br if it had not degassed, assuming that both large Cl and Br anions (1.81 Å and 1.95 Å respectively) are distributed, and behave under sufficient pressure, similarly to large ionic lithophile elements as light rare earth elements and K. Also implicit in the assumption is that Cl and Br are richer in the Iceland mantle plume relative to the depleted low velocity layer feeding

Fig. 1 Locations and elevations of basalt analysed for Cl and Br from the Reykjanes Ridge and the NE and SW Neovolcanic Zones over Iceland. Curves 1, 2 and 3 represent the predicted first-order model for Cl and Br variations along the profile (see text). DLVL-derived, LILE-depleted low velocity layer mantle source of normal ridge basalts occurring south of 61°N; plume-derived, basalts from the Iceland mantle 'plume' (see ref. 9).



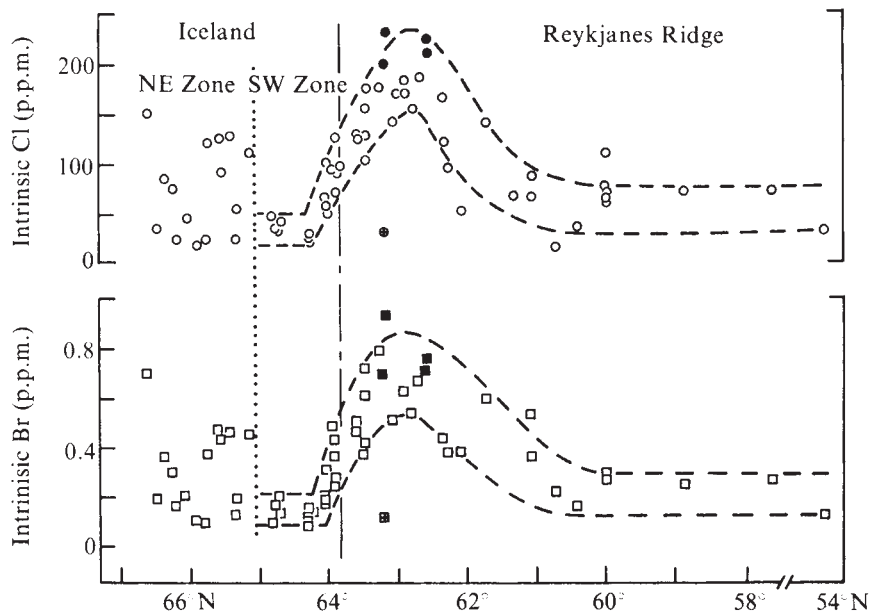


Fig. 2 Intrinsic Cl and Br variations along the Reykjanes Ridge and Iceland versus latitude. Solid symbols are samples used for calculating average values for Cl and Br maxima (see text). Cross-filled symbols are for rounded vesicular basalt (not pillow) TR101 12D40 dredged at this location, probably subaerially erupted and later submerged. Anomalously low Cl and Br contents of this sample are consistent with subaerial origin.

normal ridge segments, and that both mantles mix to produce the observed gradients⁹⁻¹¹. (See ref. 12 for a more general definition of plumes. For the physics of the phenomenon see ref. 13.)

Curve 2, on the other hand, predicts Cl and Br distribution assuming degassing for eruptions above 500 m of hydrostatic water pressure and subaerially, and that basalts are all derived from a uniform single mantle source such as beneath the subaerial and submerged flank of Kilauea volcano^{4,5,7}. This empirical degassing curve not only reflects the effects of decreasing gas solubility with decreasing hydrostatic pressure, but also the effect of vesiculation, that is the complex competition among rates of nucleation, growth, coalescence and escape of gas bubbles in a viscous medium, as well as mechanical entrapping of the bubbles, during the formation of glassy pillow rims by rapid quenching of lava against seawater^{4,6,14}.

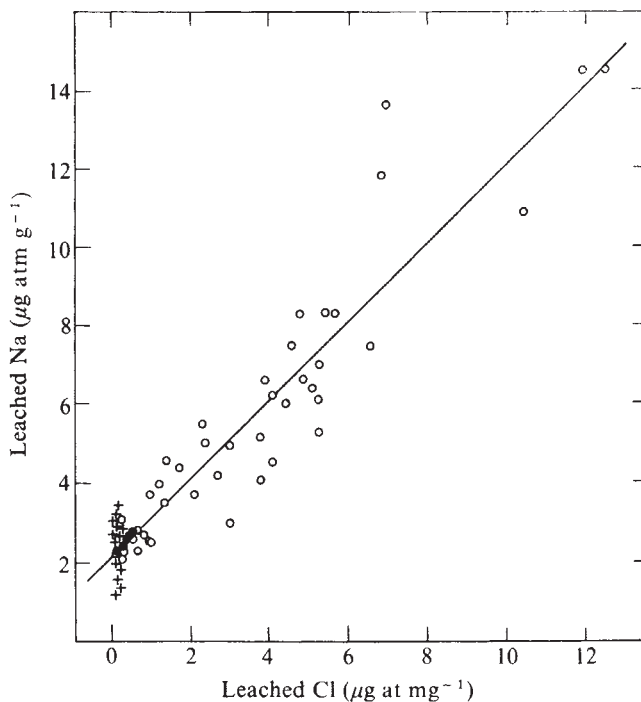
Curve 3, compounding added effects of curves 1 and 2 above, illustrates the predicted Cl or Br distribution along the Reykjanes-Iceland ridge profile. The curve reflects both processes of degassing above 500 m hydrostatic water pressure and the mixing of the Cl-rich Iceland mantle 'plume' with the Cl-poorer and LILE-depleted low velocity layer source of normal ridge basalts.

Curve 3 predicts: (1) a maximum Cl or Br concentration at the critical depth of degassing above which vesiculation occurs at an accelerating rate; (2) a constant level over Iceland, assuming that differentiation factors such as degrees of partial melting and fractional crystallisation during lava ascent are constant along the profile, or that the effect of their variations is negligible; (3) constant level south of 61°N where the 'plume' overflow beneath the ridge has become negligible^{9,15} assuming that Cl and Br are undersaturated at such depth and complicating factors of differentiation discussed above do not intervene; and (4) a transition zone along which volatile contents increase toward Iceland. Such gradient should mostly reflect the extent of the Iceland mantle 'plume' overflow beneath the ridge and relative mixing proportions of both mantle sources, as suggested by Schilling⁹, Hart *et al.*¹⁰, and Sun *et al.*¹¹. The two constant levels predicted over Iceland and south of 61°N on the Reykjanes Ridge depend mostly on the relative Cl and Br content of the two mantle sources and relative degrees of melting, as we believe that degassing of Cl and Br above 500 m of hydrostatic water pressure is relatively negligible.

Any deviation of the actual Br and Cl variations from curve 3 model prediction should help estimate the operative extent and variation of fractionation processes, such as partial melting and fractional crystallisation along the profile, the relative Cl and Br content of the two mantle sources, or any of our first order assumptions.

The actual variations of intrinsic Cl and Br we measured in basalt erupted along the Reykjanes Ridge-Iceland profile (Fig. 2) fully confirm our first-order model predictions, and implicitly justify the first-order assumptions including degassing and the Iceland mantle 'plume' and LILE-depleted asthenos-

Fig. 3 Leached Cl plotted against leached Na of the basalts studied. Note 1:1 Na and Cl variations on an atomic abundance basis. +, Subaerial basalts; ○, submarine basalts; ●, submarine glasses. $y = 2.27 + 0.98x$; $r = 0.95$.



phere mixing hypothesis. Figure 2 shows that more than 90% of the intrinsic Cl and Br content of Iceland-Reykjanes Ridge basalts, whether plotted against distance from the land-sea boundary or water depth, lies in a narrow envelope as predicted by the first-order model. Scatter along the Northern Neovolcanic Zone in Iceland is further discussed below.

The Cl and Br contents of these lavas were determined by two high precision neutron activation techniques^{16,17} on glass rims, pillow interiors and water-soluble leachates using high-purity distilled water to overcome possible Cl contamination by seasalt. Leachates of the rock powder were also analysed to ascertain that possible Cl contamination was indeed due to simple evaporation of NaCl within the cracks and vesicles of these lavas. Figure 3 shows that fresh glasses were, as expected, not contaminated, nor generally subaerial basalts, whereas relatively random amounts of NaCl addition were present in the submarine pillow basalt interiors. A plot of total Cl (or Br) in the leached rock and water leachate gave large scatter without any particular trend observable. On the other hand, as shown in Fig. 2, intrinsic Cl and Br trends (which here is defined as the Cl content of glasses, or Cl content of water leached rock powder from pillow interiors and subaerial basalts) are, to a first order, in remarkable agreement with the model prediction of Fig. 1. In the following discussion, we will consider only intrinsic Cl and Br contents. The close similarity of intrinsic Cl with that of Br along the Reykjanes Ridge-Iceland profile reinforces the validity of our first-order model, and emphasises the close geochemical similarity of Cl and Br during magmatic processes, whether it is partial melting, fractional crystallisation, volatilisation or magma degassing. A coefficient of correlation of 0.95 is obtained between Cl and Br from the 64 degassed and undegassed basalts studied. A least square fit of the data yields the relation: $\text{Br(p.p.m.)} = 0.0034 \text{ Cl (p.p.m.)} + 0.038$, for the 64 sample population studied.

We conclude that the intrinsic Cl and Br maxima at 62°40'N shown in Fig. 2 correspond to the critical depth of degassing (400–500 m depth), and the northward decrease from this depth (pressure), is predominantly caused by degassing due to the volatile property of Cl and Br. The possible nature of the mechanisms responsible for the degassing of Cl and Br above such critical depth is discussed below.

The effect of increasing hydrostatic pressure on Cl and Br content of lavas below 400 m southward along the ridge and down to about 800–1,000 m, cannot be estimated from our data. This is because the segment coincides with the transition zone of mantle mixing and the 220 p.p.m. Cl and 0.78 p.p.m. Br maxima in these lavas are much below their saturation at the time of quenching (assuming temperature of eruption of the order of 1,150–1,250 °C, based on Hermes and Schilling¹⁸, Fisk and Schilling¹⁹, and solubility data of Iwasaki and Katsura²⁰). South of 61°N, Cl, Br and H₂O (ref. 8) remain constant despite the fact that hydrostatic pressure keeps increasing due to > 1,000 m of water (Fig. 1). The observation suggests that these three volatiles, whether saturated or undersaturated above 500 m, are all undersaturated below this depth. Their concentration levels are primarily controlled by their content in the LILE-depleted asthenosphere source of these normal ridge basalts, but not by increasing hydrostatic pressure which has apparently a negligible effect below 500 m.

The finding that the fall of Cl and Br levels above 500 m and subaerially is caused by degassing is further corroborated by a similar decrease of these two elements relative to more refractory LIL elements such as K₂O and particularly La (Fig. 4). Also, the large Cl and Br scatter over the NE Neovolcanic Zone shown in Fig. 2 is considerably reduced when normalised to La or K (Fig. 4), suggesting that the scatter is due primarily to fluctuation in partial melting and fraction crystallisation rather than degassing or volatilisation.

Cl and Br degassing mechanisms

The 400–500 m critical depth of degassing of Cl and Br nearly coincides with that of H₂O, S and Se previously reported from

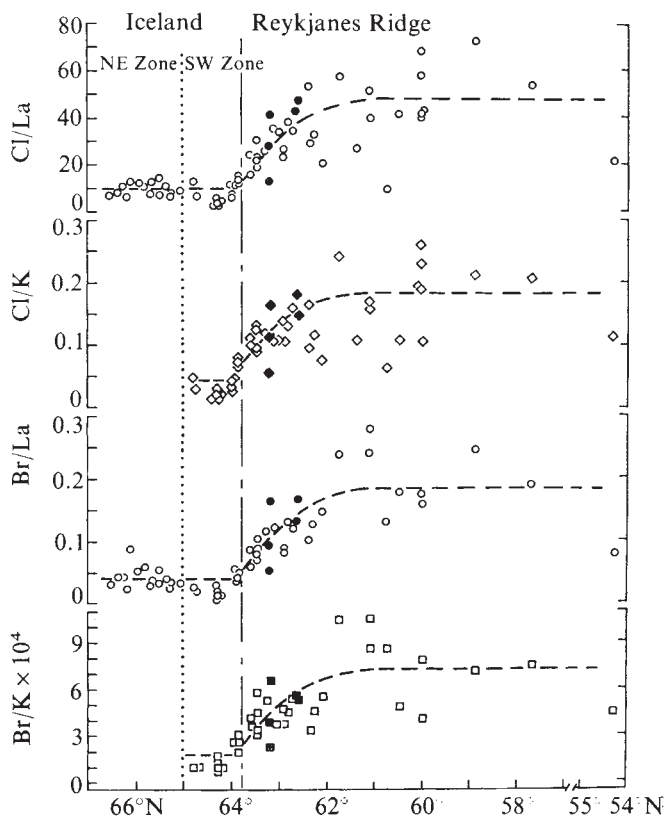


Fig. 4 Intrinsic Cl/La, Cl/K, Br/La, and Br/K versus latitude; La and K data are from ref. 9.

the same samples^{8,21}. The exsolution and rapid effervescence of all these volatiles also coincide with a rapid increase of volume percent of vesicles, reaching up to 50% of the quenched lava above this depth. The observation is indicative of the potential explosiveness of lava erupting at depths shallower than 500 m, caused by reduced confining pressure and resulting marked increase of surface area per unit volume of exposure of lava to seawater, and rapid fuel-coolant interactions²². On the basis of H₂O solubility data in basalts and thermodynamic considerations, McBirney⁶ suggested that water should drastically degas at such depth, whereas this is apparently not the case for S, Cl and Br which are either saturated (sulphur), or undersaturated (Cl and Br) at such depths and temperatures of eruptions^{8,3,20}.

Iwasaki and Katsura²⁰ showed that the solubility of Cl (as HCl) in basaltic melts is 14,536 p.p.m. at 1 atmosphere pressure of HCl, 1,200 °C and in anhydrous conditions. They also showed that the solubility of Cl increased with increasing partial pressure of HCl in the gas phase coexisting with the melt. Thus, even the maximum Cl content of 232 p.p.m. observed along the Reykjanes profile seems to be very much below HCl saturation of basaltic melts, had it been in equilibrium with an HCl gas phase only. Experimental data for Br are lacking, but analogy with HCl suggests undersaturation of Br in basaltic melts as well. Thus, based on solubility considerations alone, Cl or Br should not have exsolved from these basalts and mechanisms other than solubility must be invoked. The coincidence of critical depth of degassing of trace volatiles Cl and Br with water, which usually is the major component of volcanic gases^{3,23}, suggests that water vapour effervescing from the lava may have been responsible for the fluxing of Cl and Br out of the magma. This mechanism is further corroborated by the Cl partition data between water fluid and molten rocks determined experimentally by Kilinc and Burnham²⁴. They found that Cl favours by 31:1 the water vapour at 2–8 kbar, 700–750 °C for rocks ranging from granodioritic to granitic

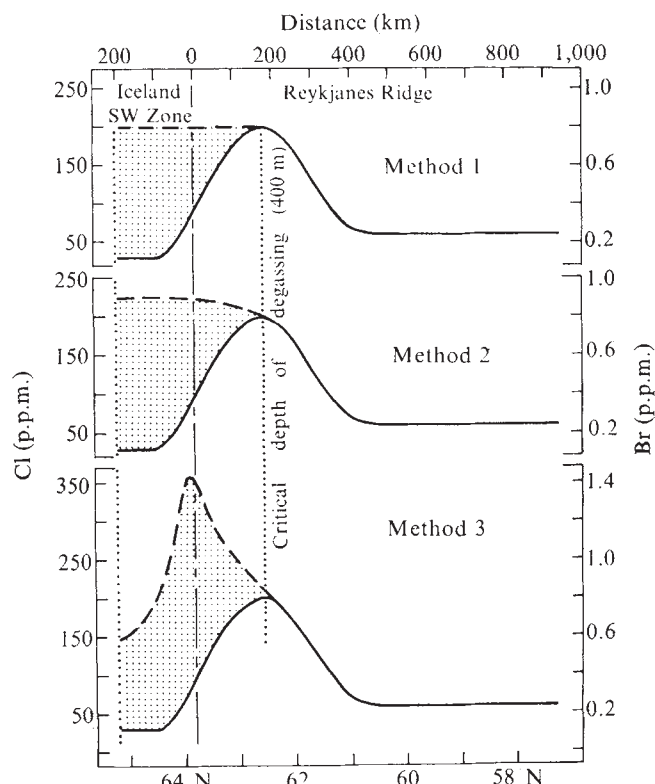


Fig. 5 Schematic diagram showing Cl (Br) loss by degassing above 400 m depth and subaerially over Iceland. Estimate of Cl (Br) flux was obtained from concentration difference observed between Cl (Br) variations (continuous line) and extrapolated values for un-degassed basalts above 400 m and subaerially erupted (dashed line), using methods (1), (2) and (3) described in text (Cl or Br flux = Cl or Br loss shown by dotted area in $\text{km p.p.m.} \times \text{lava production rate in } \text{km}^3 \text{ km}^{-1} \text{ yr}^{-1}$). Data based on Cl/La = 44; Br/La = 0.16.

composition. Using these data and Rayleigh's distillation equation²⁵, we estimate that up to 2.3% water should have been lost from the original melt to produce the observed Cl variations above 500 m and over Iceland. This is troublesome as loss of water observed along the same profile is only 0.2% maximum, as readily seen in Fig. 6 of ref. 8. Closed system gas/melt equilibrium models such as used for example by Sigvaldason and Oskarsson²⁶ (which incidentally we believe inappropriate for an apparently open system such as degassing) would only increase the discrepancy. Either the partition coefficient K_{Cl} of 31 in favour of water vapour used is in error, or another mechanism must be invoked. We calculate with Rayleigh's equation that K_{Cl} should be 256–1,151 for basaltic melts if Cl fluxing ranging from 40 to 90% of the initial Cl content were to be attributed to 0.2 weight % water effervescence only. This seems unacceptably high. Also, on the one hand, K_{Cl} values lower than the 31 experimentally determined for melts of granodioritic to granitic composition are predicted for tholeiitic melts on the basis of $\text{Na}_2\text{O}/\text{CaO}$ and its effect on degassing of Cl discussed later. On the other hand, Iwasaki and Katsura²⁰ have found that in anhydrous conditions Cl solubility (as HCl) increases with decreasing SiO_2 content of silicate melt in equilibrium with pure HCl vapour. It is not known to what extent the two opposite effects counteract each other. Clearly, experimental data are needed to resolve this question. Alternatively, it is possible that CO_2 , the second most important volatile in magmatic gases, may have played an important part in controlling the degassing of Cl and Br either during its effervescence, or as gas bubbling through shallow depth magma chambers beneath the ridge³. Unfortunately, no data on CO_2 content of the basalts and Cl partitioning between $\text{CO}_2(\text{g})$ and silicate melt are so far available to evaluate such additional

fluxing mechanism. Theoretical considerations on thermal interaction between hot lava and seawater by fuel-coolant interaction (FCI) suggest that hydroexplosions occur at depths shallower than 700 m for heterogeneous nucleation, and 130 m for homogeneous nucleation of bubbles²². However, it is not clear whether Cl or Br would have time to equilibrate during such a flash process, and as a result would increase or decrease in lavas. Whatever the exact mechanism, the near coincidence of the 500-m critical depth of degassing for S and H_2O , and now for Cl and Br, strongly suggests that minor and trace volatiles, whether saturated or undersaturated in the melt, are probably significantly flushed out by more abundant volatiles H_2O and possibly CO_2 , when effervescing after reaching saturation. This probably occurs because of the preferential partitioning of such minor and trace volatiles into the major vapour phase, relative to the basaltic melt. Complex reactions involving H_2O , CO_2 , H_2 , O_2 , H^+ , OH^- , HCl , HBr , H_2S , SO_2 , S, Cl, Br and such like species, and their gas-melt partitioning, are also likely to take place at this stage.

The critical depth of degassing seems to have important influence on magnetic properties of these lavas as it also coincides with the disappearance of well-developed magnetic lineaments^{27–29}. The disruptive effect on the melt caused by gas effervescence and bubbling, to extent of complete randomisation of grains during ash formation, tends to decrease proportionally the bulk magnetisation of such deposits if occurring at temperatures at or below the Curie points of such material, and as a result should partly blur magnetic lineaments³⁰.

Finally, it should also be noted that the 500-m critical depth for degassing of volatiles coincides with the Iceland shelf-break beyond where water depth decreases more rapidly southwards along the ridge (Fig. 1). The critical depth coincides also with a rapid increase of vesicles content and consequently decrease of density of these lavas⁸. Such foaming of lava above 500 m may have been partly responsible for the unusual build-up of submerged Iceland platform relative to the ridge, although mantle plume upwelling is probably also another independent cause⁹.

Cl and Br degassing-rate estimates

Minimum input rates of Cl and Br into the atmosphere and hydrosphere by Iceland volcanism can readily be estimated from the product of magma generation rate times the amount of Cl or Br loss above 500 m and subaerially. The magma production rate is obtained from spreading rates, whereas the amount of Cl and Br loss is obtained graphically by integrating along the ridge the Cl and Br concentration differences between undegassed and degassed basalts. Three independent approaches can be used to calculate the amount of Cl and Br loss, as illustrated in Fig. 5. These are (1) by simply extrapolating over Iceland the observed Cl and Br maxima northwards of the critical depth of degassing by assuming that the concentration of Cl and Br maxima is representative of the initial undegassed lava before eruptions; or (2) by extrapolation northwards of the critical depth the calculated Br and Cl content of undegassed plume-derived basalts on the basis of proportion of the binary mantle mixture obtained from Sr and Pb isotopic gradients^{10,11}; or (3) by extrapolating the observed Cl and Br curves northwards of their maxima by considering constant Cl/La and Br/La ratios, assuming a close geochemical coherence between La and Cl or Br during partial melting and fractional crystallisation processes occurring at pressures well above the partial and vapour pressures of Cl and Br. Our estimates of the minimum input rate of Cl due to volcanism along the Reykjanes Ridge and Middle Neovolcanic Zone on Iceland is $0.58\text{--}5.61 \times 10^3$ metric ton yr^{-1} by the first method, $0.66\text{--}6.38 \times 10^3$ ton yr^{-1} by the second method, and $0.79\text{--}6.68 \times 10^3$ ton yr^{-1} by the third method. The corresponding figures for Br are 1.82–18.18, 2.4–20.8 and 2.83–24.76 ton yr^{-1} respectively. The uncertainties in our estimates are directly and largely influenced by the uncertainties in values used for lava production rates which vary

between 6×10^{-6} and $65 \times 10^{-6} \text{ km}^3 \text{ km}^{-1} \text{ yr}^{-1}$ along the profile^{27,31,33}.

The above estimates can be doubled if the other northern half of Iceland, including the Tjornes Fracture Zone, is also considered. Thus, our total input of about $1 \times 10^4 \text{ ton yr}^{-1}$ of Cl from Iceland volcanism compares favourably with the estimate of $2.9 \times 10^4 \text{ tons yr}^{-1}$ of Cl, as HCl, given off by Kilauea volcano in Hawaii³³. Decker³⁴ and Bargar and Jackson³⁵

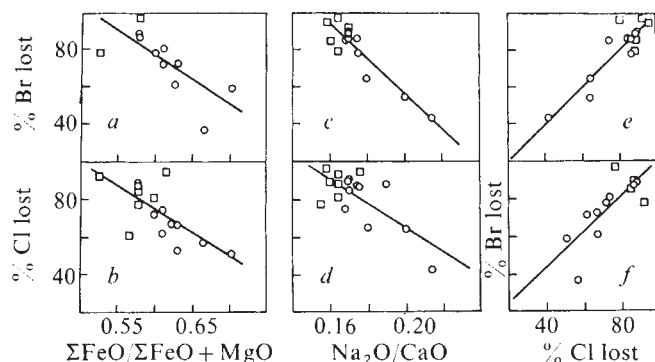


Fig. 6 Plot of %Cl and %Br lost by degassing obtained from differences between degassed and undegassed lava pairs with equal $\Sigma\text{FeO}/\Sigma\text{FeO} + \text{MgO}$ (a, b) or $\text{Na}_2\text{O}/\text{CaO}$ (c, d) ratios, versus the corresponding $\Sigma\text{FeO}/\Sigma\text{FeO} + \text{MgO}$ and $\text{Na}_2\text{O}/\text{CaO}$ ratios. Symbols used to illustrate locations of lava pairs are: $\circ$, degassed Iceland basalt—undegassed basalt from the region of Cl and Br maxima; $\square$, degassed Iceland basalt—undegassed basalt from the region South of Cl-Br maxima. Also, note the 1:1 correspondence between %Cl and %Br lost from lava pairs with equal $\text{Na}_2\text{O}/\text{CaO}$ (e) and $\Sigma\text{FeO}/\Sigma\text{FeO} + \text{MgO}$ (f) ratios. Lines drawn in (a) to (f) are least square best fits.

have shown that both Iceland and Hawaiian volcanic edifices have similar lava production rates, thus adding further credibility to our independent approach.

Discrepancies from first-order model and comparison with other results

The previous degassing estimate suggests that subaerial basalts from Iceland lose 40% to 90% of their original Cl and Br. Smaller amounts are observed down to 500 m of water, and no degassing apparently occurs below this depth (Fig. 2). On the other hand, comparison of this trend with that empirically predicted in Fig. 1 suggests that a significant discrepancy occurs on the Reykjanes Peninsula on Iceland. Complete degassing would have been expected over this region as further inland. The discrepancy may either be attributed to the possibility that these lavas were previously erupted beneath the sea, or by the effect of melt composition on extent of degassing. The first explanation is ruled out since the lava we sampled comes from flows having typical subaerial surface morphological forms which do not resemble pillow lava sequences usually found on the ocean floor. The second explanation is suggested from the fact that $\Sigma\text{FeO}/\Sigma\text{FeO} + \text{MgO}$ and $\text{Na}_2\text{O}/\text{CaO}$ and other major elements change slightly but significantly inland toward central Iceland^{18,36}. Figure 6 shows that both Cl and Br per cent losses by degassing over Iceland are sensitive inverse functions of the bulk composition of the lavas expressed either as Fe/Mg or Na/Ca ratios. Mechanistically, we suggest that this reflects an increasing tendency of Cl (and Br) to form non-volatile complexes such as NaCl or related bonds in the melt with increasing alkalinity of the melts, that is, increasing Na activity; thus reducing the escaping tendency of Cl and Br from the melt during effervescence of other major volatiles. By extrapolation of such curves in Fig. 6, we estimate that subaerial alkali-rich lavas with $\text{Na}_2\text{O}/\text{CaO}$ ratios greater than 0.3 would not lose any appreciable Cl and Br during eruptions. Sigvaldason and Oskarsson²⁶

have suggested that tholeiitic basalts on Iceland lose only 10% of their original Cl eruption. This is in disagreement with our general finding along the Reykjanes Ridge and S W Neovolcanic Zone on Iceland, where we estimated that 40–90% of the initial Cl of the lavas is lost before quenching. These two diametrically opposed views can be reconciled in the case of basalts having alkalic affinities and $\text{Na}_2\text{O}/\text{CaO}$ ratios greater than about 0.30; but this is not the case for the tholeiitic basalts reported here. Independent evidences are required to resolve the dispute as both approaches are indirect.

Cl and Br contents of Iceland 'plume' and LILE-depleted asthenosphere

Such estimates can readily be obtained from average estimates of Cl and Br contents of basalts erupted south of 61°N on the normal ridge segment (60 p.p.m. and 0.22 p.p.m. respectively), and from our previous estimates of Cl and Br content of undegassed tholeiite over Iceland (222 p.p.m. and 0.78 p.p.m. respectively). We also assume that tholeiitic basalts along the profile are produced over a small range of partial melting averaging approximately 25–30%³⁷, and that most of the Cl and Br diffuse into the interstitial melt during partial melting in the mantle. In such a case, we obtain by simple material balance a Cl content of 61 ± 6 p.p.m. and Br 0.21 ± 0.02 p.p.m. for the mantle 'plume' beneath Iceland, and a Cl content of 17 ± 2 p.p.m. and Br content of 0.06 ± 0.01 p.p.m. for the LILE-depleted asthenosphere. Thus, the Iceland mantle 'plume' seems to be three to four times richer and more pristine in Cl and Br than the source of normal ridge basalts.

We conclude that the Cl and Br variations reported here not only confirm our first-order model for degassing of such volatiles along the Reykjanes Ridge–Iceland profile, but also give further support to the mantle 'plume' hypothesis, as its effect on Cl and Br variations was an implicit part of our first-order model prediction.

Further implications of these findings, such as on the origin of Cl and Br of seawater, will be discussed elsewhere (see also ref. 36).

We thank F. DiMeglio and his staff for neutron irradiations and facilities at Rhode Island Nuclear Science Center; P. Aldrich for typing; and Rannsóknarad Ríkisins (Icelandic Research Council) for permission to carry out this research on Iceland territory. This work was supported by the US NSF.

Received 31 August; accepted 16 November 1977.

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Palaeoclimatic and geomorphic implications of $^{230}\text{Th}/^{234}\text{U}$ dates on speleothems from Britain

T. C. Atkinson

University of East Anglia, Norwich, UK

R. S. Harmon

Michigan State University, East Lansing, Michigan 48824

P. L. Smart

University of Bristol, Bristol, UK

A. C. Waltham

Trent Polytechnic, Nottingham, UK

A priori arguments and empirical evidence both suggest that widespread deposition of calcite in caves takes place in non-glacial climatic conditions. Radiometric dates from calcite speleothems in Britain indicate deposition before 170,000 yr b.p., during an interglacial around 90–140,000 yr b.p., an interstadial at 60,000 yr b.p. and in the late Devensian and Holocene. The positions of speleothems within caves allow minimum ages to be estimated for past water tables and associated surface landforms. The main erosion of the Yorkshire Dales is shown to date from before 400,000 yr b.p., while Cheddar Gorge in the Mendip Hills has been deepened by 70 m during the last 400 millennia.

SPELEOTHEMS are mineral formations found in limestone caves. They have been classified on the basis of mineralogy and form, calcite stalactites, stalagmites and flowstones being the commonest¹. All three forms usually display a structure of fine growth layers when sectioned and polished, with major hiatuses in deposition or other changes in depositional environment being marked by erosional surfaces, dirt bands and sometimes colour changes.

In the British Isles, cave systems up to several tens of kilometres in length have been explored, in areas underlain by the Carboniferous Limestone, most notably in north-west Yorkshire, the Peak District in Derbyshire, South Wales, the Mendip Hills near Bristol and large areas of Ireland. The age of these caves has hitherto been unknown but they have generally been presumed to have formed during the Pleistocene^{2–4}. We have

obtained 28 $^{230}\text{Th}/^{234}\text{U}$ dates from 21 samples of stalagmites and flowstones from caves in north-west Yorkshire and the Mendip Hills. These dates throw new light on the age of the caves themselves and on the age of some of the erosional features of the landscapes beneath which they have formed. They also provide an indication of the timescale of climatic events during the late Pleistocene in Britain.

The basic principle of the $^{230}\text{Th}/^{234}\text{U}$ method as applied to speleothems is that isotopes of U leached from the rock above the cave will be co-precipitated as uranyl carbonate with the calcite of the speleothem. The precipitating solution will normally be free of Th, as the Th^{4+} ion is strongly adsorbed onto clay minerals or precipitated as insoluble hydrolysates. Thus, provided that it includes no clay or other insoluble detritus, a freshly formed calcite speleothem will contain between 0.01 and 100 p.p.m. of U, and no Th. The ratio of ^{234}U to its daughter ^{230}Th can be used to estimate the age of ancient speleothems. A disadvantage of the method is that ^{230}Th has a much shorter half life than ^{234}U so that an isotopic equilibrium is eventually set up in which the decay of parent and daughter proceeds at the same rate. This constraint limits the method to samples younger than 400,000 yr.

Application of the $^{230}\text{Th}/^{234}\text{U}$ disequilibrium method to dating speleothems requires four essential conditions. (1) The sample should contain sufficient U for analysis. (2) The initial isotopic state of the system must be known. This is achieved by rejecting samples containing more than 1% acid-insoluble detritus. (3) The sample should have remained closed to isotopic migration throughout its history, a condition met by rejecting samples which show any evidence of recrystallisation or partial dissolution. (4) The observed $^{230}\text{Th}/^{234}\text{U}$ ratios should

Table 1 Uranium concentrations, isotope ratios and calculated ages of British speleothems

Sample Code	Cave	[U] (p.p.m.)	$\frac{^{230}\text{Th}}{^{234}\text{U}}$ *	$\frac{^{234}\text{U}}{^{238}\text{U}}$ *	$\frac{^{230}\text{Th}}{^{232}\text{Th}}$ *	Calculated age (10^3 yr b.p.)	Description and location within cave
GB1A	G.B. Cave, Mendip Hills	23.6	0.096 ± 0.050	2.07 ± 0.03	52	11 ± 6	Stalagmite: outer shell: White Passage } †
GB1B	G.B. Cave, Mendip Hills	1.2	0.070 ± 0.010	2.40 ± 0.04	29	8 ± 1	Stalagmite: inner core: White Passage } †
GB1C	G.B. Cave, Mendip Hills	0.25	0.045 ± 0.021	2.19 ± 0.06	> 1,000	< 5	Stalagmite: outer shell: Balcony } †
GB1D	G.B. Cave, Mendip Hills	0.18	0.46 ± 0.10	2.11 ± 0.09	114	63 ± 19	Stalagmite: inner core: Balcony } †
GB1E	G.B. Cave, Mendip Hills	0.22	0.12 ± 0.03	2.56 ± 0.09	25	13 ± 3	Stalagmite: upper shell: upper terrace, Bridge } †
GB9B	G.B. Cave, Mendip Hills	0.11	0.01 ± 0.07	1.15 ± 0.07	118	> 360	Flowstone: upper layers: 10 Foot Pot } †
GB17:1	G.B. Cave, Mendip Hills	0.14	0.995 ± 0.090	1.07 ± 0.05	29	> 390	Flowstone: basal layers: Top of Gorge } †
GB20:1	G.B. Cave, Mendip Hills	0.17	0.735 ± 0.08	1.71 ± 0.14	20	127 ± 25	Stalagmite: outer layer: Mud Passage } †
GB23A	G.B. Cave, Mendip Hills	0.05	0.47 ± 0.06	2.41 ± 0.44	13	63 ± 11	Flowstone: basal layer: upper terrace, Gorge } †
GB24	G.B. Cave, Mendip Hills	0.06	1.00 ± 0.20	1.22 ± 0.27	> 1,000	332 ± 100	Flowstone: basal layer: middle terrace, Gorge } †
GB27	G.B. Cave, Mendip Hills	0.11	0.84 ± 0.05	1.36 ± 0.08	501	172 ± 26	Stalagmite: bulk: Bat Passage } †
GB29	G.B. Cave, Mendip Hills	0.27	0.08 ± 0.02	2.49 ± 0.09	> 1,000	9 ± 2.5	Stalagmite: bulk: Bat Passage } †
GP2	Gavel Pot, Yorkshire	0.85	0.13 ± 0.07	1.27 ± 0.03	15	14 ± 9	Stalagmite: middle layers: Glasfurd's Passage } †
GP3	Gavel Pot, Yorkshire	0.39	0.053 ± 0.011	1.22 ± 0.04	> 1,000	6 ± 1	Stalagmite: basal layers: Glasfurd's Passage } †
Y1	Gavel Pot, Yorkshire	0.28	0.725 ± 0.052	1.33 ± 0.06	57	131 ± 18	Stalagmite: centre layers: Glasfurd's Passage } †
Y3:1	Gavel Pot, Yorkshire	0.19	0.44 ± 0.02	1.41 ± 0.03	> 1,000	61 ± 4	Stalagmite: outer layers: Glasfurd's Passage } †
Y3:2	Gavel Pot, Yorkshire	0.13	0.595 ± 0.031	1.49 ± 0.03	20	92 ± 5	Stalagmite: middle layers: Glasfurd's Passage } †
Y3:3	Gavel Pot, Yorkshire	0.15	0.675 ± 0.019	1.55 ± 0.03	92	112 ± 5	Stalagmite: basal layers: Glasfurd's Passage } †
Y5	Gavel Pot, Yorkshire	0.76	0.105 ± 0.005	0.83 ± 0.01	16	12 ± 1	Flowstone: top layers } †
KM1	Kingsdale Master Cave, Yorks.	0.28	1.06 ± 0.06	1.20 ± 0.05	> 1,000	> 400	Flowstone: bulk: Roof Tunnel } †
WS1	White Scar Cave, Yorks.	1.06	0.105 ± 0.010	1.14 ± 0.03	33	12 ± 2	Stalagmite: basal layers } †
WS2	White Scar Cave, Yorks.	0.22	0.12 ± 0.05	1.64 ± 0.15	17	13 ± 7	Stalagmite: outer layers: Main Streamway } †
WS3:1	White Scar Cave, Yorks.	0.35	0.023 ± 0.020	2.19 ± 0.05	> 1,000	< 5	Stalagmite: outer layers: Battlefield } †
WS3:2	White Scar Cave, Yorks.	0.40	0.053 ± 0.024	1.99 ± 0.08	45	6 ± 2	Stalagmite: middle layers: Battlefield } †
WS3:3	White Scar Cave, Yorks.	0.35	0.60 ± 0.05	2.27 ± 0.14	100	90 ± 11	Stalagmite: basal layers: Battlefield } †
WS5	White Scar Cave, Yorks.	0.38	0.93 ± 0.07	1.29 ± 0.07	50	225 ± 75	Stalagmite: outer layers: Main Streamway } †
WS6:1	White Scar Cave, Yorks.	0.21	0.13 ± 0.06	2.60 ± 0.20	17	14 ± 7	Stalagmite: outer layers: Main Streamway } †
WS6:2	White Scar Cave, Yorks.	0.24	0.14 ± 0.03	2.02 ± 0.17	38	16.5 ± 4	Stalagmite: basal layer: Main Streamway } †

*Isotope activity ratios.

†Brackets denote serial samples on a single speleothem.

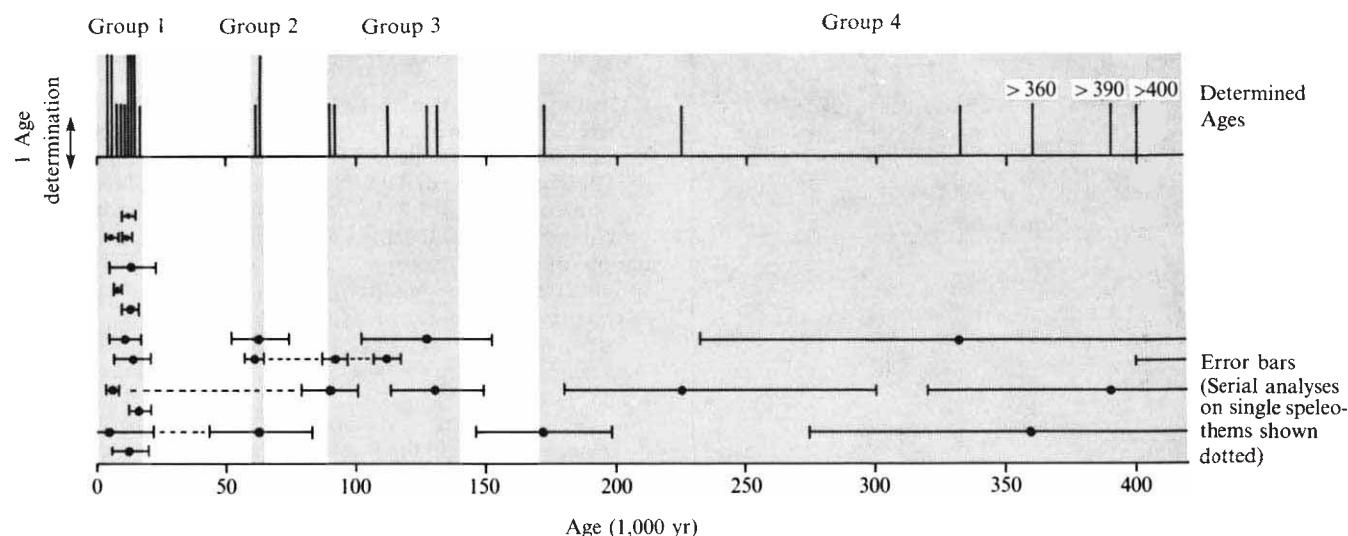


Fig. 1 Distribution of age determinations of British speleothems.

be in correct stratigraphic order, that is, decreasing from the bottom to top of the specimen. As far as can be judged these four criteria were met in all but one of the samples analysed.

Analytical procedures used were similar to those described by Thompson⁵. A 50–100-g sample of speleothem was dissolved in 2M HNO₃ and filtered if any detrital material was present. A ²²⁸Th/²³²U tracer and Fe³⁺ carrier were added, allowed to equilibrate with the solution, and then the radionuclides coprecipitated with Fe(OH)₃. After washing with distilled deionised water the precipitate was dissolved in 9M HCl and iron removed by extraction into isopropyl ether. U was then separated by ion exchange on Dowex 1-XB resin in a chloride form and Th by separation on the same resin in a nitrate form. Further purification of the U and Th was accomplished by extraction into a 0.2 M TTA/benzene solution at a pH of 1.2 for Th and 3.5 for U.

²³⁸U, ²³⁴U, ²³²U, ²³²Th, ²³⁰Th, and ²²⁸Th isotope activities were measured for each sample immediately after evaporation onto stainless steel disks by α -particle spectrometry. The counting system involved a silicon surface-barrier detector and low noise amplifier, with an Ortec 6240 multichannel analyser. Peak energies were routinely monitored with a ²⁴¹Am source, the ²³⁰Th peak centering at 4.7 MeV and the ²³⁴U peak at 4.2 MeV. Approximately 8,000 counts were recorded under the principal U and Th peaks. Background activity was low, in the order of 0.1 c.p.m. but was monitored and corrected over the energy range of each isotope peak on a fortnightly basis. Background corrected ²³²Th activities were negligible, but 5% of the ²²⁴Ra activity had to be subtracted from that of its parent ²²⁸Th because of the overlap in decay energies of these two isotopes.

Measured U concentrations, U and Th isotope activity ratios, and calculated ages are given in Table 1. The ages were calculated from the background- and yield-corrected ²³⁰Th/²³⁴U and ²³⁴U/²³⁸U ratios on a program modified from Thompson⁵, using the general equation

$$[^{230}\text{Th}/^{234}\text{U}]_t = \left(\frac{1 - \exp(-\lambda_{230}t)}{[^{234}\text{U}/^{238}\text{U}]_t} \right) + \left(\frac{\lambda_{230}}{\lambda_{230} - \lambda_{234}} \right) \times \left(1 - \frac{1}{[^{234}\text{U}/^{238}\text{U}]_t} \right) \times \left(1 - \exp(-(\lambda_{230} - \lambda_{234})t) \right)$$

where $[^{230}\text{Th}/^{234}\text{U}]_t$ and $[^{234}\text{U}/^{238}\text{U}]_t$ are the measured activity ratios at time t and λ_{230} and λ_{234} are the decay constants of ²³⁰Th and ²³⁴U respectively. The age uncertainty quoted is derived from the 1 σ uncertainty in the counting statistics. In certain cases this figure is large because of the low U concen-

trations of the speleothem material analysed. In all but three cases U concentrations are less than 1 p.p.m. resulting in low count rates and relatively large analytical uncertainties in isotope activity ratios, in spite of the 30–60% chemical yields. Only one specimen (GB 17:2, not included in the data set) contained more than 1% detrital insoluble material. In all but one case where multiple age determinations were made on the same specimen the ages were in correct stratigraphic order. The ages for the Holocene sample GB1A/B were in reverse stratigraphic order, but the large uncertainty of 50% in the age of GB1A overlaps the small uncertainty in GB1B.

Palaeoclimatic implications

Speleothems are deposited from water containing dissolved Ca²⁺ and carbonate species, which has percolated through the limestone above the cave roof. Two mechanisms govern precipitation. First, the water may be evaporated. This process is sometimes important in the entrance zone of caves and has also been recorded deep within caves in the semi-arid south-western USA⁶. Deep within British caves, however, the relative humidity is normally close to 100% (refs 7, 8) and degassing of carbon dioxide is the main mechanism of calcite deposition. This process occurs because percolating waters which have passed through soil or been in contact with decaying organic matter in the unsaturated zone usually have a partial pressure of CO₂ exceeding that of the cave atmosphere. Degassing of CO₂ occurs when the water enters the cave causing it to become supersaturated with calcite. This process has been verified in the field by chemical analysis of water samples^{6,9}. Thus speleothem deposition seems to require two necessary conditions; a supply of percolating water and a reservoir of CO₂-rich gas from which the water may acquire a higher P_{CO_2} than cave air. Both these conditions are met in Britain at present and field studies show that calcite supersaturation of percolation waters is normal in caves in the Mendip Hills^{10,11}. However, there are good reasons for supposing that speleothem deposition ceased, or was at least drastically reduced, during past periods of periglacial and glacial conditions.

Glacier melt waters and snow melt waters analysed by Ford¹² in the Canadian Rocky Mountains have a P_{CO_2} between one half and one hundredth of the atmospheric value of 10^{-3.6} atm at around 2,000 m above sea level. Glacier melt waters from Iceland have P_{CO_2} of 10^{-5.5} to 10^{-4.5} atm (ref. 13). Karst springs draining meltwaters from beneath the Columbia ice-field in the Canadian Rocky Mountains have P_{CO_2} between 10^{-3.3} and 10^{-5.0} (ref. 12). Similarly, streams draining areas of tundra vegetation in the same area have P_{CO_2} between 10^{-3.7} and 10^{-4.2}. Thus, evidence of present processes in alpine

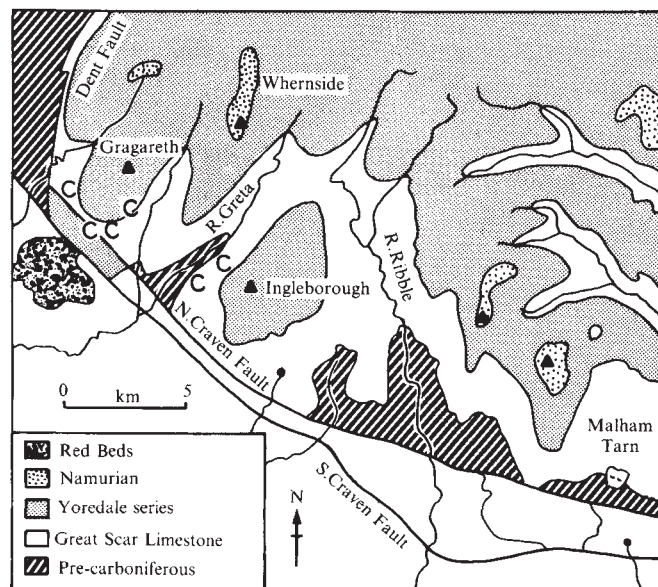


Fig. 2 Outline geology of the Ingleborough area, north-west Yorkshire. C, cave site included in Fig. 3.

environments suggests that percolating groundwaters would not have contained enough CO_2 for degassing and speleothem deposition to have occurred in caves during periglacial or full glacial conditions in Britain. Chemically less detailed studies of limestone solution in the Canadian Arctic support this conclusion^{14,15}.

A second reason why speleothem deposition would probably be prevented or slowed during periglacial conditions is that percolation would almost totally cease in areas of continuous permafrost. The extent of permafrost in Britain during the Devensian may be inferred from relict features. While it was probably widespread in northern England it is not certain whether permafrost occurred in the area around the Mendip Hills¹⁶.

Figure 1 is a plot of the speleothem age data. The ages fall into four groups: (1) A late Devensian and Holocene period of speleothem deposition beginning around 17,000 yr b.p. and continuing to the present; (2) a short mid-Devensian episode around 60,000 yr b.p.; (3) a longer period of deposition from about 90,000 to 140,000 yr b.p.; (4) early 'episodes' of uncertain duration before 170,000 yr b.p. These periods were probably times when the climate was not periglacial.

Gascoyne¹⁷ has published preliminary $^{234}\text{U}/^{230}\text{Th}$ dates for three speleothems from north-west Yorkshire which support

this interpretation. One is Holocene, one falls into our 90,000–140,000 yr b.p. period, and the fourth has an age of 37,000 yr b.p.

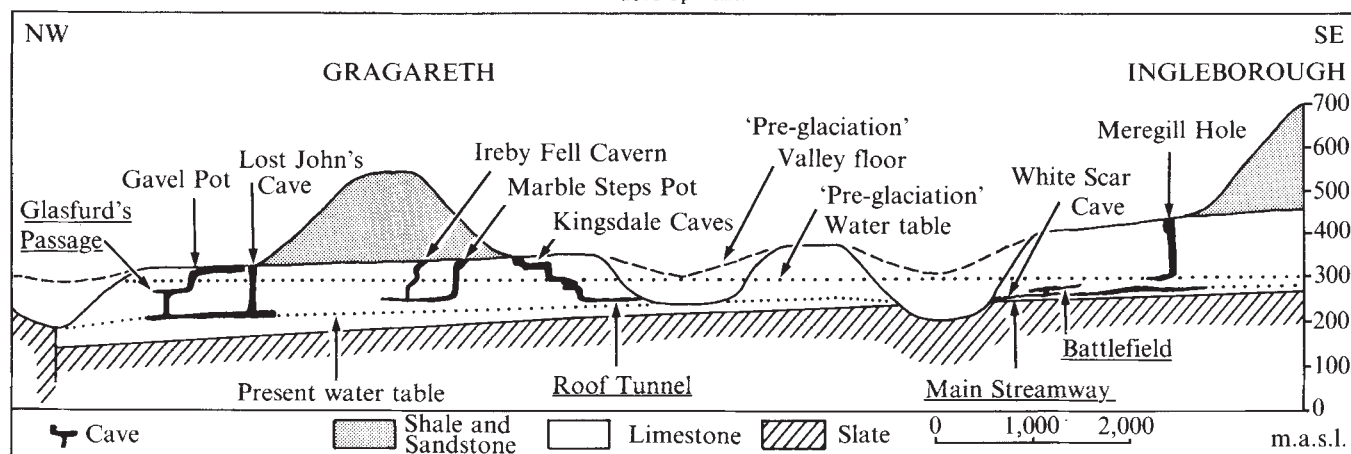
On the basis of the distribution of age determinations and the generally accepted Upper Pleistocene stratigraphy of Britain, we correlate the 60,000 yr b.p. depositional event with the Chelford interstadial and Stage 3 of the marine foraminiferal oxygen isotope record^{18–20}. The 90,000–140,000 yr b.p. period probably represents the Ipswichian Interglacial and stage 5 of the marine oxygen isotope record. Gascoyne's¹⁷ 37,000 yr b.p. specimen may represent part of the complex Upton Warren interstadial recognised by Coope¹⁸.

The periods of non-deposition of speleothems occurring between the groups shown in Fig. 1 agree well in age with similar periods determined for North American speleothems^{21,22}. The latter were based on over 70 radiometric age determinations including serial analyses of single stalagmites²³. In a study of speleothems from alpine North America, Harmon²⁴ recognised interglacial periods of speleothem deposition at 330–290 Kyr, 240–190 Kyr, 125–100 Kyr, approximately 60 Kyr and 15 Kyr–present.

Geomorphic implications

The area around Ingleborough Hill in north-west Yorkshire (Fig. 2) is one of Britain's most spectacular karst areas. Its caves have been described by Waltham and others³ and its geomorphology by Sweeting²⁵, Clayton²⁶ and Waltham²⁷. Sweeting and Clayton recognise fragments of a high-level erosion surface at about 400 m above sea level. This surface cuts across the geological structure in several places but has been shown to be structurally controlled by nearly horizontal strata in others²⁷. It coincides in places with the top of the Great Scar Limestone, which forms a bench at altitudes of 330–430 m around the major hilltops which are composed of Yoredale Series shale and sandstone. On the basis of river profiles, Sweeting²⁵ recognises two stages of rejuvenation beneath this surface. The first seems to have produced valley floors at around 210–270 m while a later rejuvenation was responsible for the steep river profiles where the rivers Ribble, Greta and Doe cross the Craven Faults (Fig. 2). While Sweeting considers glaciation to have had only a minor effect in sculpting the present land surface, other authors, notably Clayton²⁶, suggest that the main valley rejuvenation was due to glacial erosion. In the adjacent area of the Forest of Bowland, Moseley²⁸ has detected three erosion surfaces in the present landscape. The Bowland Surface lies between 360 and 560 m above sea level and may correlate with the 400 m surface in the Ingleborough district. The Upper Wyresdale Surface is represented by the upper slopes of valleys incised into the Bowland Surface at 130–310 m and probably correlates with the first rejuvenation

Fig. 3 Diagrammatic section through Ingleborough and Gargareth, showing sample locations (underlined) and inferred landscape development.



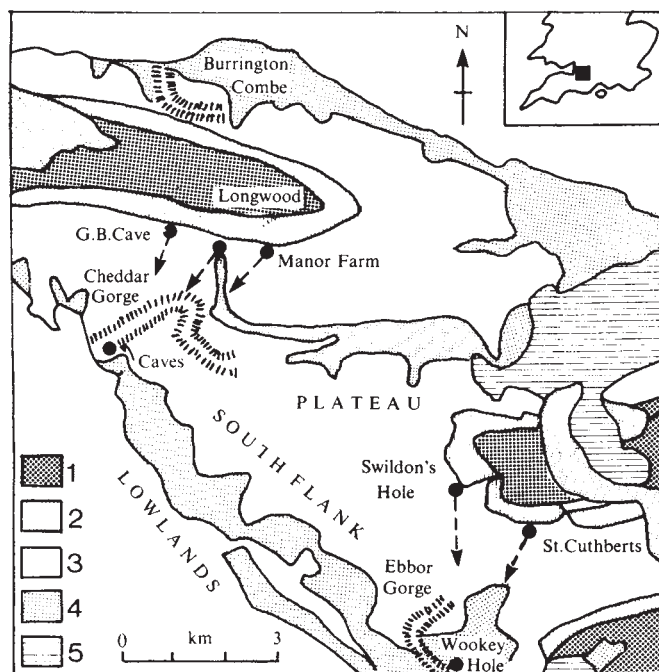


Fig. 4 Outline geology of part of the Mendip Hills. 1, Devonian sandstone; 2, Lower Carboniferous shale; 3, Carboniferous limestone; 4, Triassic breccia; 5, later rocks.

at Ingleborough. Finally, the Lower Wyresdale Surface has a base level at about 70 m above sea level and represents a further stage of rejuvenation.

In cave passages a variety of features have been recorded which can be used to establish whether the cave was originally formed by solution above the water table (vadose caves) or below it (phreatic caves)²⁹. In Yorkshire two classes of cave passage can be distinguished: (1) large phreatic tunnels fed by vadose inlets, both often containing inwashed sediment and glacial debris; (2) clean, sediment-free vadose passages feeding flooded phreatic tunnels below the modern water table, which in turn feed the springs which at present drain the limestone. A previous position of the water table can be identified at places where passage features indicate a change from vadose to phreatic origin. Although the history of individual cave systems is complicated, it is possible to identify a high-level past water table in all of the major systems transected by the diagrammatic section in Fig. 3. The features identifying this water table all occur in the first class of caves. The ancient water table occurs above 265 m above sea level close to the Dent Fault in the north-west and at over 300 m on the flank of Ingleborough in the south-east. It lies up to 85 m above the present floors of the major valleys.

The ancient phreatic caves were apparently drained fairly swiftly by a major rejuvenation. In several cave systems there is evidence of a second past water table, followed by a second rejuvenation of 7–20 m to the position of the modern water table. We suggest that the 265–300-m water table dates from a time when the floors of the major valleys lay at about this height. The magnitude of the first rejuvenation in the caves is as great as 75 m and its apparent swiftness suggests that the cause was rapid valley deepening by glacier erosion. If this were the case then the valley floors between 265 and 300 m above sea level were probably pre-glacial. Subaerially formed speleothems in the passages drained by rejuvenation would then provide minimum ages for the glaciation which was responsible for the valley deepening.

The two oldest dated speleothems from north-west Yorkshire are samples KM 1 and WS 5. The former is a flowstone from a phreatic passage at 260 m above sea level which was drained when the resurgence of the Kingsdale Master Cave reached its

present level at 249 m. The determined age of greater than 400,000 yr b.p. suggests that the main valley deepening was accomplished by an early glaciation before this date. The date of 225,000 yr b.p. for WS 5, a stalagmite from the vadose main stream passage of White Scar Cave, is consistent with this. White Scar Cave is formed in strata just above the base of the Carboniferous Limestone and we may infer that the Greta valley had been eroded down to this level before this speleothem was deposited.

The Mendip Hills, 25 km south of Bristol, present a contrast to north-west Yorkshire. Here the karst area consists of a plateau at about 250 m above sea level separated from surrounding lowlands by steep flanking slopes. A well-developed dry valley network is incised into the plateau which is largely underlain by steeply dipping Carboniferous Limestone (Fig. 4). The dry valleys debouch into the lowlands through deep gorges of which the Cheddar Gorge is the best known. Springs flow from the limestone at the mouths of the gorges and fossil effluent caves up to 70 m above the springs have been studied in detail^{30,31}. Streams which drain sandstone and shale hills rising above the 250 m plateau flow into caves which have also been studied^{32–34}. In the case of the caves whose streams feed the Cheddar spring, past water tables have been identified which correlate with fossil effluent caves in the walls of Cheddar Gorge at 92, 62, and 40 m above sea level (Fig. 5).

In one of the engulfment caves, G.B. Cave, we have dated the speleothems listed in Table 1 and related them to the cave's erosional history³⁵. The results show that G.B. Cave was drained to a local water table level of 135 m by 360,000 yr b.p. (samples GB 9B and GB 17:1). This water table level is correlated on stratigraphic and geomorphic grounds with Great Oones Hole, a fossil phreatic effluent cave in Cheddar Gorge at 92 m above sea level. We may infer, therefore, that underground drainage was well-developed by about 360,000 yr b.p. at which time the mouth of Cheddar Gorge lay at about 90 m above sea level, 160 m below the plateau surface.

In all probability the Mendip Hills were unglaciated during the Pleistocene but there is strong evidence³⁶ that they lay very close to an ice front during at least one glaciation. The dry valleys and gorges are thought to have formed partly by normal fluvial erosion before underground drainage developed and partly during periglacial periods when ground ice may have prevented underground drainage. High-level phreatic caves above the altitude of Great Oones Hole attest to the early development of underground drainage. Thus, the depth of the early incision of Cheddar Gorge suggests that the region experienced lengthy periods of cold climate before 360,000 yr b.p.

In both of the areas studied speleothem age determinations provide clear evidence that karst scenery was well developed before 350,000 yr b.p. In both areas also, major valleys had been incised to considerable depths by the same date. In the Mendip Hills, which are close to the coast, a decline in local base level of some 70 m during the Upper Pleistocene may be inferred from the altitudinal difference between fossil resurgences active over 360,000 yr ago and the present springs at Cheddar.

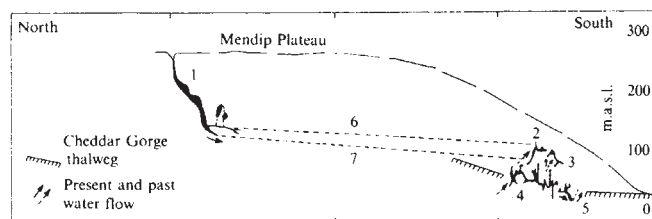


Fig. 5 Diagrammatic section from G.B. Cave to Cheddar Gorge. 1, G.B. Cave; 2, Great Oones Hole; 3, Long Hole; 4, Gough's Cave; 5, present active cave (largely flooded) and spring; 6, inferred position of water table between G.B. Cave and Great Oones Hole; 7, inferred water table between G.B. Cave and Long Hole.

We thank the Department of Geology, Michigan State University for facilities for speleothem dating.

Received 25 July; accepted 30 November, 1977.

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Three-dimensional structure of the lipovitellin-phosvitin complex from amphibian oocytes

Douglas H. Ohlendorf, Richard F. Wrenn & Leonard J. Banaszak

Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St Louis, Missouri 63110

Microcrystals of the lipoprotein-phosphoprotein complex which are found in the oocytes of Xenopus laevis were examined using electron microscopy. Analysis of Fourier transforms of the images of the (010) and (001) projections showed the space group to be $P2_122_1$. Ten projections were combined to produce a map of the complex having about 20 Å resolution. The lipoprotein complex consists of two subunits related by a local twofold symmetry axis. The density was averaged around the local symmetry and reasonably well defined structural domains can be seen in the resulting model.

In spite of the importance of membrane proteins and other lipoprotein complexes in biochemistry, very little is known about their molecular structure. Such complexes are generally not found in a form which is sufficiently homogeneous and ordered for study by diffraction methods. In the case of membrane proteins, the exceptions are bacteriorhodopsin contained in the purple membrane patches from *Halobacterium halobium*¹ and cytochrome oxidase isolated from the inner membrane of mitochondria². The structure of bacteriorhodopsin was determined to about 7 Å resolution by methods which combine electron diffraction and imaging from two-dimensional arrays of this membrane protein¹. The structure of cytochrome oxidase was obtained to a resolution of about 30 Å using mainly electron imaging and digital reconstruction of reconstituted particles contained in negatively stained flattened vesicles. In both cases, the lipid content of the membrane-like system is about 20 to 25% w/w.

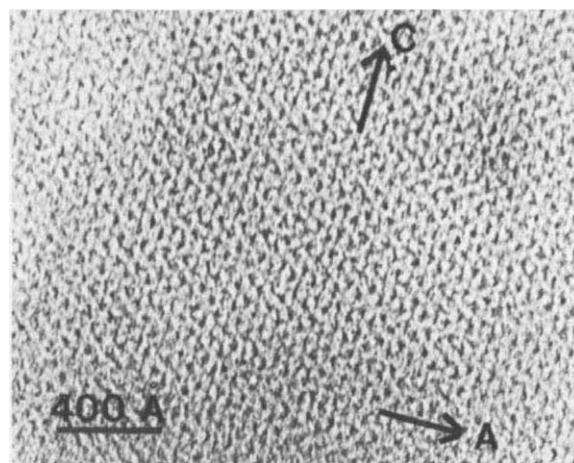
Other forms of lipoprotein complexes are soluble in aqueous systems. Typical examples of soluble complexes are the high-density serum lipoproteins which contain 45-65% w/w of lipid³. Models of this class of lipid-protein systems have been determined by small angle X-ray scattering methods⁴⁻⁶. The resulting models generally consist of a spherical lipid core about 90 Å in diameter surrounded by a shell about 10-20 Å thick thought to consist mainly of the polypeptide components and phospholipid head groups⁴⁻⁶. Even in the case of the high density serum lipoproteins which are relatively monodisperse, variations in the amount and type of lipid suggest that the preparation of crystals for structural studies will be difficult.

A crystalline lipoprotein complex occurs *in situ* in the yolk from amphibian oocytes⁷⁻⁹. This complex consists of two proteins, a lipoprotein called lipovitellin and a phosphoprotein called phosvitin. These proteins are synthesised in the liver as a precursor

called vitellogenin, which then binds about 12% w/w lipid¹⁰. Vitellogenin is transported in the serum to the ovaries where it is proteolytically cleaved into phosvitin and lipovitellin and stored as microcrystals called platelets¹¹. In the yolk system of *Xenopus laevis*, lipovitellin contains 22% lipid—approximately 75% of which is phospholipid, predominantly phosphatidylcholine, and the rest is neutral lipid. Lipovitellin is dimeric and each monomer contains three different polypeptide chains of molecular weights 105,500, 35,500, and 32,000 (ref. 12). Phosvitin is believed to be a single polypeptide chain of molecular weight 17,000 in which over 50% of the amino acids are phosphoserine¹². In total, the yolk complex consists of the dimeric lipovitellin and two molecules of phosvitin with a total molecular weight of 456,000 (ref. 12).

Because of the crystalline nature of the yolk system, it is possible to obtain a relatively detailed model of the lipoprotein including its overall size and shape, the arrangement of subunits in the dimeric protein and some information about the structural domains within a single subunit. The model described here was obtained from a series of electron micrographs of negatively stained crystalline fragments using three-dimensional image reconstruction^{13,14}.

Fig. 1 An electron micrograph of a yolk crystal fragment after staining with uranyl acetate. The view which is shown is the (010) projection with the direction of the A and C axis as indicated.



Electron microscopy and image reconstruction methods

Bright-field electron micrographs of the microcrystals negatively stained with uranyl acetate are obtained at typical magnifications of 100,000 (ref. 15). The regions of each electron micrograph in which the lattice is most clearly seen are examined using optical diffraction. The optical diffraction patterns are then used to evaluate: (1) the degree of order in the micrograph, (2) the electron microscope contrast transfer function, and (3) the orientation of the lattice with respect to the electron beam¹⁶. Preliminary identification of the fragment orientation is based only on the lattice spacings. About 90% of the several hundred electron micrographs screened are of the (010) projection which is shown in Fig. 1. This view has a pseudosquare symmetry which suggests that there might be a local twofold axis orientated approximately perpendicular to the **B** axis at $\pm 45^\circ$ to the **A** axis. Nine other lattice views are identified among the electron micrographs. Significant among these is the (100) projection which was absent from the views previously reported¹⁵.

Preliminary attempts at subjectively reconstructing a three-dimensional model of the crystalline lipoprotein were made with optically filtered electron micrographs. These first attempts were unsuccessful largely because of ambiguous interpretations which must be made in combining image projections. In contrast, the digital method of three-dimensional image reconstruction does not suffer from any such arbitrary interpretations¹³ and is used in this structural study as follows. The optical density of the most highly ordered regions in the electron micrographs of the lattice view measured with an Optronics P-1000 densitometer on a $25 \times 25 \mu\text{m}^2$ raster. Next the Fourier transform of each projection is calculated and a two-dimensional lattice is ascribed to the peak positions in the calculated transform using a method of least squares. The sampling interval around each peak in the calculated Fourier transform is then decreased by the application of a digital interpolation filter¹⁷ such that the transform near the peak positions is sufficiently oversampled (five to six times) to make linear interpolation accurate. The amplitudes at reciprocal lattice points are determined by integrating under each peak of the now oversampled Fourier transform. The phase at a reciprocal lattice point is determined by linearly interpolating the complex Fourier transform to the reciprocal lattice point defined by the least squares procedure. The contrast transfer function of each electron micrograph is determined from the background in the calculated and optical diffraction patterns and is used to correct both the magnitudes and phases of the reciprocal lattice terms¹⁴.

Before the projections can be combined into a three-dimensional map, the phases of the structure factors from each view must be adjusted to a common origin and the amplitudes placed on a common scale. As the reflections in the (010) and (001) projections are centrosymmetric, origins can be determined by minimising the r.m.s. phase differences from centric values of the stronger reflections in each plane. Because there are eight possible origins in the orthorhombic unit cell, the phases of three reflections uniquely specify a single origin. The phase relationships observed in these two projections also show that the space group is $P2_122_1$. In the case of non-centric zones, the adjustment of phases to a common origin is more complicated. The relative phases of symmetry-related reflections are determined by the space group. Hence if $h'k'l'$ is symmetry-related to hkl where $h' = \pm h$, $k' = \pm k$, $l' = \pm l$, relationships can be found which are of the form

$$\alpha_{hkl} \pm \alpha_{h'k'l'} = 0^\circ \text{ or } 180^\circ$$

where α_{hkl} is the phase of the hkl reflection. Using a method of least squares, an origin is found which best satisfies these relationships for the stronger reflections. If ambiguous results are obtained for a given projection, that origin is selected for which the resultant phases of common reflections best agree with the previous estimates. If the identification of a lattice view based on spacings obtained from optical diffraction is ambiguous, the

identification selected is the one for which the phases best agree with previously determined values.

Several electron micrographs of the (010) projection are averaged and used in the reconstruction. In this averaging the magnitudes of the reflections to $35 \text{ \AA}$ are used for scaling. For dissimilar views, methods for combining amplitudes based solely on the ratios of common reflections produce conflicting scale factors. Therefore the other nine projections plus the averaged (010) projection are scaled by first defining a matrix **G** such that G_{ij} is the sum of the magnitudes of the reflections in the i^{th} projection which are common to the j^{th} projection. Scale factors, c_j are chosen such that the matrix **H**, where $H_{ij} = c_j G_{ij}$, is as nearly symmetric as possible. Multiple estimates of the scaled real and imaginary parts of the same reflection which arise from different views are averaged.

Using the individual phase estimates and the final averaged values, r.m.s. phase errors, ϵ_α , can be calculated. Among the centric reflections which are greater than three times the estimated standard deviation in amplitude, $\epsilon_\alpha = 28.8^\circ$, while for all non-zero reflections, $\epsilon_\alpha = 49.6^\circ$. These phase errors compare favourably with $\epsilon_\alpha = 104^\circ$ which correspond to the case in which the phase estimates are random. They are in fact similar to $\epsilon_\alpha = 39^\circ$ obtained from the centric $hk0$ zone of catalase crystals but are not as accurate as phases obtained from purple membrane patches where $\epsilon_\alpha = 18^\circ$ (ref. 18). The better values are probably due to the absence of negative stain and to the use of low dose electron microscopy.

Three-dimensional maps

The 10 electron micrographs which are used in the three-dimensional reconstruction define all of the observable reflections to a resolution of $35 \text{ \AA}$ and should have included 419 out of the 586 unique reflections to a resolution of $15 \text{ \AA}$. However, because of experimental difficulties, only a total of 305 independent reflections are determined. Finally, although measurable reflections to $13 \text{ \AA}$ are determined for the (010) projection, in many other zones the resolution is not as good so that the overall resolution of the resultant map is estimated to be slightly less than $20 \text{ \AA}$. Interestingly, in evaluating the resolution limits, the X-ray powder diffraction methods have shown that the microcrystalline lipoprotein complex remains highly ordered even after lyophilisation in the presence of uranyl acetate¹⁵.

The adjusted phases and scaled amplitudes are then used to calculate a three-dimensional map of the negatively stained unit cell at intervals of **A**/16, **B**/32, **C**/32 $\text{\AA}$. A clearly defined molecular envelope is followed throughout most of the map. However, at several positions along the crystallographic twofold axes parallel to **B**, the density appears continuous, that is, it crosses the symmetry element. As previous estimates based on the dimeric nature of lipovitellin indicate that there are four molecules per unit cell, it is felt that the density situated on the twofold axes is due to the resolution limits of the electron micrographs and that the molecular centres of the complex are probably located in general positions in the unit cell. The density along the twofold axes which is visible in the three-dimensional map can also be seen in the original electron micrographs.

Starting with a region of the map where the molecular envelope is clearly defined, a model is constructed following the best connectivity. It then becomes apparent that regions of the density are related by a local twofold symmetry axis and this fact is consistent with the extra symmetry seen in the (010) projection contained in Fig. 1. Using the local symmetry and the regions of each subunit where the molecular envelope is best defined, it is possible to assign segments of density that appear to be continuous throughout the map. The envelope of a single lipoprotein complex from the native map is shown in Fig. 2 looking approximately down the local twofold axis. As can be seen in this stereo drawing, the lipoprotein complex appears similar to most dimeric proteins in that there is twofold rotational symmetry between the component subunits.

To further improve the low resolution map, the structure is averaged around the local symmetry element. The position of this

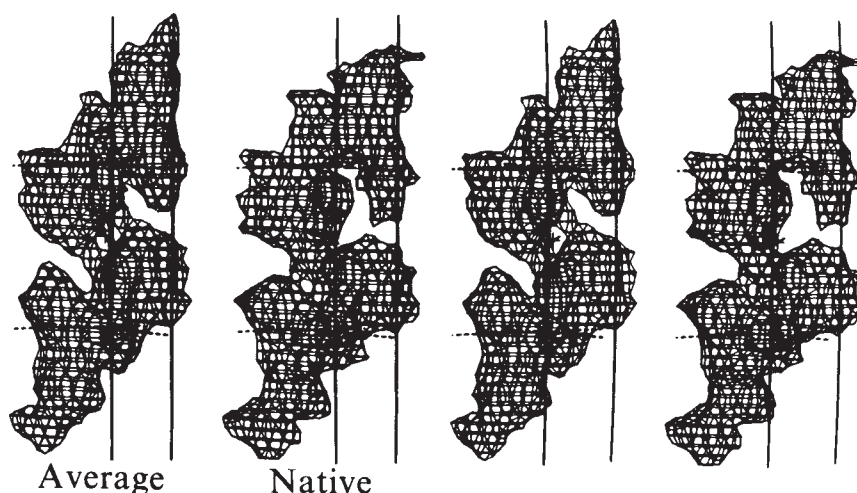


Fig. 2 Stereo drawings of the molecular envelope of the lipovitellin-phosvitin complex. The contoured region is the selected asymmetric unit from a three-dimensional map obtained by using the method of image reconstruction as described in the text. The complex shown on the right is from the native map and that on the left is from the map averaged around the local twofold axis. Maps have been contoured at 13% of the highest positive peak on the protein map. The vertical lines and the dashed lines are the crystallographic twofold and twofold screw axes, respectively. The viewing direction is 5° above the local twofold axis, which is shown by the solid line appearing to go into the page. The drawings were produced on a MSS-X computer display system.

axis is found by maximising the correlation coefficient, r , between the protein density and that obtained after the local twofold rotation. The correlation coefficient is

$$r = \frac{\sum_{xyz} \rho \rho_r}{\sum_{xyz} \rho^2}$$

where ρ is the protein density at any point x, y, z in the map and ρ_r is the density at the same point after the trial twofold rotation. The final position of the local twofold axis is perpendicular to the **B** axis at an angle of -52° with the **A** axis, passes through a point having the fractional coordinates (0, 0.464, 0.5), and results in a correlation coefficient of 0.63. Final averaging of the protein density map is done by first changing values in the solvent region to zero and then averaging around the local twofold axis with a computer program written by Dr P. Bethge which used Bricogne's algorithm¹⁹.

A stereo drawing comparing the averaged and native maps is shown in Fig. 2. Most of the distinguishing features of the native structure are unaltered after imposing the local symmetry. Furthermore, examining a difference map between the native and averaged structures shows that there are no systematic errors in the averaging process. In addition, as a further check on the reliability of the averaging procedure, the image 'structure factors' are calculated from the averaged protein map and compared with those from the original map with the solvent region set to zero. The resulting R factor as described in Table 1 is 0.44. The r.m.s. phase difference between the observed image structure factors and those obtained from the averaged protein map is 67.4° . Based on these internal checks, it was concluded that the averaged protein map is a better estimate of the overall structure and it is used throughout the remainder of the discussion.

Low-resolution structure of lipovitellin-phosvitin complex

The lipovitellin-phosvitin complex seems to be a somewhat elongated but flattened structure which could be roughly described by an ellipsoid of approximate dimensions, $55 \times 115 \times 250$ Å. Two views of the low-resolution model from the averaged map are shown in Fig. 3. Using the map, one can calculate that the volume of the lipoprotein complex is $550,000$ Å³, its surface area is $22,000$ Å² and its radius of gyration is 51 Å. From the previously reported chemical composition¹² and partial specific volumes, a monomeric unit of lipovitellin and phosvitin should have volumes of $268,000$ Å³ and $20,000$ Å³, respectively. Hence the volume of the calculated structure of the complex is only about 5% smaller than the expected value. At the contour level used in Fig. 3, the solvent content would be about 30% (v/v) which, although somewhat low, agrees with the value reported by Wallace¹⁹. The tight packing of molecules in the unit cell which is

suggested by this low solvent content is also evidenced by the stability of the microcrystals even in the absence of water¹⁵.

Examination of the structure of the complex (Fig. 3) reveals several features. First, it should be noted that the contact between the subunits is selected as shown because of a distinct puckering in the surface, particularly near the molecular twofold axis and the structural element labelled A. In addition the protein density immediately around the local twofold axis is weak, almost producing a hole. This is a typical feature around symmetry elements associated with oligomeric proteins.

Although not easily visible in Fig. 3, the surface structure of the lipoprotein complex shows the presence of several distinct domains—labelled A, B, C, L, and P in Fig. 3. The domains are characterised by distinct ridges and depressions much like those near the subunit contact region. Note that the L domain is only seen on the surface visible in the view labelled 'front'. The approximate volumes of domains A, B, C, and P, assuming ellipsoidal shapes, and the approximate area of domain L are given in Table 2. From these volumes one can calculate the size of the protein which might fit into each domain. Domains A, B and C could contain peptides of molecular weight 100,000, 34,000 and 25,000. As the three polypeptides, LV-A, LV-B, and LV-C present in lipovitellin have molecular weights of 105,500, 35,500 and 32,500 (ref. 12), it is tempting to assign them to domains A, B,

Table 1 Image reconstruction data from the lipovitellin-phosvitin complex

Unit cell dimensions ¹⁶	$a = 88.6$ Å $b = 172.4$ Å $c = 196.1$ Å
Space group	$P2_122_1$
No. of projections	10
No. of unique reflections observed	305
Nominal resolution	20 Å
r.m.s. phase error (e_x)*	49.6°
R factor† after averaging	0.44

*The r.m.s. phase error is defined as

$$e_x = \left[\frac{(1/N) \sum_{i=1}^N (\alpha_i^{\text{obs}} - \alpha_i^{\text{calc}})^2}{1} \right]^{1/2}$$

where the sum is only over the phase estimates of reflections which are centric or are common to two projections. α_i^{obs} is the phase estimate from the Fourier transform of a projection. α_i^{calc} is the average phase for each reflection after correcting the origin and imposing the space group symmetry.

†The R factor is defined in the usual crystallographic manner,

$$R = \frac{\sum_{hkl} \|F_{hkl}^{\text{obs}}\| - |F_{hkl}^{\text{calc}}|}{\sum_{hkl} |F_{hkl}^{\text{obs}}|}$$

where F_{hkl}^{obs} and F_{hkl}^{calc} are the observed and averaged image structure factors obtained from a map with the solvent region set to zero.

and C, respectively. However, such assignments are clearly arbitrary and based only on the rough volume estimates.

Because phosvitin, the phosphoprotein member of the complex, can be readily dissociated from the dimeric form of lipovitellin²⁰, it is equally tempting to assume that it is related to the structural domain labelled P in Fig. 3. This region seems to form a small cap on the C domain and is almost totally surrounded by solvent. The volume of the P domain would accommodate a protein of molecular weight about 6,000. Phosvitin has only a few hydrophobic residues and, therefore, it might well have a fairly open conformation which could allow the uranyl acetate negative stain to interpenetrate. Based on its exposure to solvent and the relatively small volume, the region labelled P could be tentatively assigned to phosvitin. This being the case, the remainder of the structure must be the lipovitellin and the principal intra-subunit contacts of lipovitellin are then made by the regions labelled A.

As the solvent content of the crystals is relatively low, it is not surprising that there are several intermolecular contacts. These are numbered 1–11 in Fig. 3 with a prime symbol used to denote pairing. For example, dotted region 1 is in contact with 1' of an adjacent molecule in the crystalline lattice. Only the contact regions 8–11 do not have mates. They are in contact with the identical portion of an adjacent molecule across the crystallographic twofold axis at (0, y, 0.5) for contacts 8 and 9, and across the twofold axis at (0.5, y, 0.5) for regions 10 and 11. It is these twofold contacts which appear relatively large and consequently were troublesome in the initial interpretation of the map.

As pointed out above, the assignment of the known polypeptide components to clearly marked structural domains in the map is arbitrary and based solely on the size of the domains. One such region clearly visible on both the native and averaged map is labelled L in Fig. 3. It is characterised by a distinct thickening producing a bulge in the view labelled 'front' in Fig. 3. As it has been shown that each monomeric unit of lipovitellin contains approximately 50 lipid molecules, it is of importance to pose

Table 2 Properties of lipovitellin–phosvitin structure

Dimer	
Radius of gyration	51 Å
Surface area	22,000 Å ²
Volume	550,000 Å ³
Domains in a monomeric unit	
A	V ≈ 120,000 Å ³
B	V ≈ 40,000 Å ³
C	V ≈ 30,000 Å ³
P	V ≈ 7,000 Å ³
L	A ≈ 1,200 Å ²

questions about alternative locations for this lipid. Certainly a model consisting of a spherical core of lipid in some manner surrounded by protein can be ruled out by the dimensions of the complex alone. Because of the complex structure of the lipoprotein, it would seem equally implausible to have the lipid binding sites dispersed uniformly over the surface of the lipovitellin.

One is led to the postulate that each subunit of lipovitellin probably contains a single lipid binding domain. A corollary of such a postulate is that only a relatively small number of the lipid molecules are bound directly to protein. Additional binding would then occur by secondary lipid to lipid interactions. One way to accommodate lipid in the aforementioned manner into the low resolution structure of lipovitellin would be in the L domain. In the direction perpendicular to the views shown in Fig. 3, this L region is over 50 Å thick, adequate to accommodate an extended phospholipid molecule even with a narrow base or cover of protein on one side. It has a surface area of about 1,200 Å² again suitable for about 50 molecules of lipid.

The L domain, if it is indeed the lipid region, would be characterised by the following properties. Primary lipid binding sites would be present in each of the three structural domains A, B and C. If these domains are unique polypeptide chains as has been suggested, each chain would have only a limited number of lipid binding sites. Protein–protein contacts would still occur as is seen in Fig. 3 where domain A is in contact with B and C as well as L, and so on. The L domain in subunit I is also unique in that its surface in the view labelled 'front' is not in close contact with other molecules in the crystalline lattice. This is in agreement with preliminary observations which show that some lipid may be extracted from the lipovitellin–phosvitin complex without disturbing the crystalline lattice²¹. If the L domain is the lipid binding region, it should have no contact with the solvent except through the phospholipid head groups which would be on the 'front' of the complex. This would mean that the domain A would make a small but definite contact with B and C in the same view. The suggestion that lipid is present in the L domain is in agreement with the chemical observation that lipovitellin can be dissociated to a monomeric form without loss of its lipid binding capacity²².

In summary, the three-dimensional map of the lipovitellin–phosvitin complex is clearly interpretable in terms of four molecules per unit cell. A single unique molecular envelope is found and the resulting structure contains what seems to be a local twofold axis, which is in agreement with the fact that lipovitellin is dimeric. Furthermore, the protein map which is based on negatively stained micrographs is of sufficient resolution that subunits and even structural domains within the lipoprotein are discernible. The final model is based on an averaged protein map and this map is in good agreement with the experimental data. Tenuous but reasonable assignments of the known protein and lipid components to the different structural domains have been made. The lipoprotein itself appears much the same as other globular proteins and in no way resembles the spherical structures proposed for serum lipoproteins.

We thank M. L. Collins for her contributions in the early stages of this study. This work was supported by grants from the US NIH (GM-13925) and NSF (P-CM 76-81481).

Received 28 November 1977; accepted 9 January 1978.

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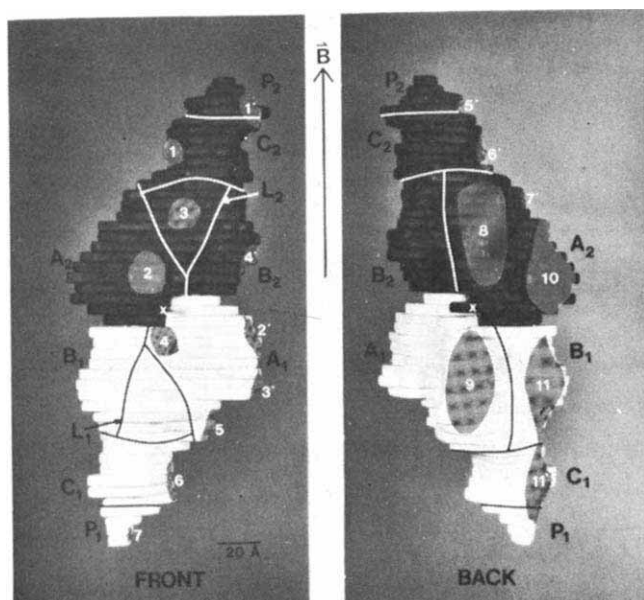


Fig. 3 Model of the averaged lipovitellin–phosvitin complex. The model was constructed from a density map where the first contour level is slightly below that shown in Fig. 2. Both views are looking down the molecular twofold axis with the two views related by 180° rotation around a vertical line. For referral, the view on the left has been labelled 'front' while that on the right is called 'back'. The arrow indicates the direction of the crystallographic B axis. Solid lines and letters divide the structure into several different domains that are discernible in the model. The dotted areas and their numbers indicate the regions of intermolecular contacts in the crystal lattice and are described in more detail in the text. The white 'x' indicates the position of the local twofold axes as used for averaging.

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In vivo synthesis and properties of uracil-containing DNA

Huber R. Warner & Bruce K. Duncan*

Department of Biochemistry, University of Minnesota, St Paul, Minnesota 55108

T4 bacteriophage DNA containing as much as 30% of its thymine replaced by uracil can be synthesised in Escherichia coli deficient in both dUTPase and uracil-DNA glycosidase. This uracil-containing DNA is competent for RNA transcription, and can be packaged into phage which are viable, if the host cells are deficient in uracil-DNA glycosidase activity. If the host cells are not deficient in this glycosidase activity the infecting phage DNA is rapidly attacked, resulting in more than 50% acid-solubilisation of the DNA. The infected cells are inefficiently killed, presumably because of very limited, if any, expression of the phage DNA. These results indicate that this replacement of thymine by uracil in DNA does not seriously impair the biological functionality of T4 DNA, provided the DNA is not subjected to the breakdown (repair) pathway initiated by uracil-DNA glycosidase.

ALL known living cells contain thymine in their DNA, but it is not clear why uracil, which forms base pairs as well with adenine as does thymine, would not suffice. Although the thymine-uracil dichotomy could have a role in discrimination between DNA and RNA, the major conformational differences between DNA and RNA are due to the presence of the 2'-hydroxyl group on the sugar moieties of RNA¹. The sugar is apparently responsible for the different interaction of RNA and DNA with some proteins, such as nucleases. The replacement of thymine by uracil in DNA leads to less thermal stability of the DNA since deoxyuridine base stacks less well than thymidine², but the wide range of thermal stabilities of DNA in various organisms suggests that the thermal stability of the DNA is not biologically significant. Thus, although the presence of uracil in the DNA might not be structurally important, the occurrence of thymine in the DNA of all known living cells suggests some biological basis for the exclusion of uracil from DNA. Hochhauser³ has recently reviewed several possible explanations for the exclusion of uracil from cellular DNA, but the question remains unresolved. To study this question it would be useful to be able to synthesise uracil-containing DNA and determine the ability of this DNA to function *in vivo*.

At least two mechanisms are known whereby the presence of uracil in DNA is precluded. The first is the presence of dUTPase activity⁴ which hydrolyses dUTP to dUMP thereby keeping the ultracellular dUTP pool low and providing the substrate for thymidylate synthetase. The second is the presence of uracil-DNA glycosidase activity⁵ which removes uracil from single and double stranded DNA should it occur.

Hochhauser and Weiss⁶ have isolated dUTPase-deficient *Escherichia coli* mutants (*dut*), and these are viable. The observation that the *dut* mutations are identical to *sof* (short Okazaki fragments) mutations⁷ provides indirect evidence that uracil is

being incorporated into DNA in these mutants, but that base excision repair leads to its removal. This repair could presumably be initiated by the uracil-DNA glycosidase activity⁸.

Duncan *et al.*⁹ have isolated uracil-DNA glycosidase-deficient *E. coli* mutants (*ung*), and these mutants are viable. *Dut⁻ ung⁻* double mutants, if viable, would presumably contain uracil in their DNA since the excision repair might not be initiated in the absence of the glycosidase activity. We have detected the presence of uracil in the DNA of such mutants (in preparation). We report here direct evidence for the incorporation of uracil into bacteriophage DNA in such mutants and we describe some of the properties of these uracil-containing DNA phage.

Synthesis of uracil-containing T4 phage

Bacteriophage have long provided a useful tool for studying nucleic acid metabolism in bacteria. In these experiments we have made use of the multiple T4 phage mutant (56⁻, *denA*, *denB*, *alc10*). Normally, T4 DNA contains hydroxymethylcytosine instead of cytosine, but 56⁻ mutants synthesise cytosine-containing phage DNA since gene 56 codes for the T4-induced dCTPase-dUTPase activity¹⁰. This gene must be mutated for the purpose of this experiment to delete the dUTPase activity. The *denB* gene codes for T4 endonuclease IV (ref. 11), which must be mutated to prevent the degradation of the cytosine-containing phage DNA which is synthesised by phage harbouring gene 56 mutations. The *denA* gene codes for T4 endonuclease II (ref. 12), which is required for degradation of the host DNA. The *alc* gene codes for a protein which prevents transcription of late genes in cytosine-containing phage DNA¹³. For convenience we will refer to these as mutant phage.

The wild-type and mutant phage were grown in various *E. coli* in the presence of [6-³H]uridine, and the DNA in the progeny phage was analysed to determine the amount of radioactivity in

Table 1 Incorporation of [6-³H]uridine into T4 DNA

<i>E. coli</i> genotype	Phage	% Radioactivity			
		H	C	T	U
W3110 (<i>dut⁺ ung⁺</i>)	Wild type T4	33	<1	66	1
BW212 (<i>dut-11 ung-1</i>)	Wild type T4	38	<1	61	1
W3110 (<i>dut⁺ ung⁺</i>)	T4 (56 ⁻ <i>denA denB alc10</i>)	9	24	66	<1
BW212 (<i>dut-11 ung-1</i>)	T4 (56 ⁻ <i>denA denB alc10</i>)	9	30	42	19

The *E. coli* were grown to about 5×10^8 cells ml⁻¹ in Fraser's medium¹⁷ supplemented with 0.1% yeast extract, 100 µg ml⁻¹ deoxyadenosine and 20 µg ml⁻¹ thymidine. The cells were washed once in Fraser's medium and infected at a multiplicity of infection of 4–5 in Fraser's medium supplemented with 0.1% yeast extract. The infected cells were labelled from 8 to 90 min after infection in the presence of 4 µCi and 1 nmol [6-³H]uridine per ml. The phage produced were purified by CsCl gradient centrifugation, and the base composition of the T4 DNA was determined after hydrolysis of the DNA for 30 min at 170 °C in formic acid¹⁸. When cytosine was subjected to this analysis procedure about 0.5% of the cytosine was deaminated to uracil; hence, we consider values up to 1% to be unreliable. *E. coli* W3110 is the *thy⁻* parent of *ung-1*. *E. coli* BW212 was constructed by B. Weiss by transduction of the *dut-11* marker into *E. coli* BD10 which carries the *ung-1* marker and was produced by treatment of *E. coli* W3110 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The residual activities of dUTPase and uracil-DNA glycosidase in *E. coli* BW212 measured *in vitro* are 1% (ref. 7), and less than 1%, respectively.

*Present address: Microbiology Department, Johns Hopkins University Medical School, Baltimore, Maryland 21205

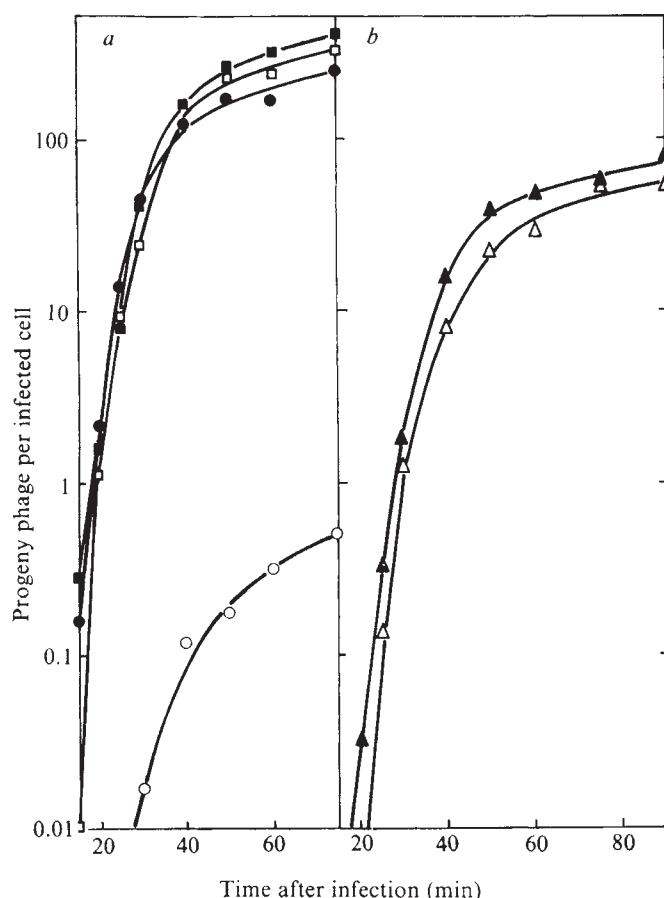


Fig. 1 Replication of uracil-containing phage. *E. coli* W3110 (*ung*⁺) and BD10 (*ung*⁻) were infected with T4-T and T4-U phage at both high (a) and low (b) multiplicity of infection. The infected cultures were shaken vigorously at 37 °C, and were diluted 2.5×10^3 at 25 min after infection to prevent reinfection of cells by progeny phage. The infected cells were lysed with chloroform at various times and the phage were titred on *E. coli* CR63 (*ung*⁺), so only uracil-free phage would produce plaques. The multiplicities of infection were 7 for *E. coli* W3110 infected with T4-T (●), 8 for *E. coli* W3110 infected with T4-U (○), 15 and 0.7 for *E. coli* BD10 infected with T4-T (■, ▲), and 17 and 0.8 for *E. coli* BD10 infected with T4-U (□, △), respectively. The titre of infected cells was assumed to be equal to the initial cell titre for infections at high multiplicity, and equal to the plaque-forming units added for infections at low multiplicity.

the pyrimidine bases (Table 1). Whereas essentially no uracil is found in T4 DNA made in *dut*⁺*ung*⁺ *E. coli*, uracil is found in DNA made in the *dut*⁻*ung*⁻ host. Since the ratio of the radioactivity in hydroxymethylcytosine (H) and thymine (T) in the wild-type T4 phage reflects the known base composition of T4 DNA (67% AT, 33% GC), the per cent radioactivity in the DNA of the mutant phage is probably a fairly reliable estimate of the base composition of this DNA. If so, about 30% of the thymine has been replaced by uracil in this DNA. For convenience we will refer to these uracil-containing phage as T4-U and the uracil-free phage as T4-T. These phages presumably carry the same genetic information.

The results shown in Table 2 compare the base composition of the total DNA in the infected cell with that of the DNA actually packaged into the phage. There is no significant difference between these two populations, indicating that the DNA actually packaged is representative of the total DNA synthesised in the infected cell. Thus, if the intracellular DNA is heterogeneous with respect to the amount of uracil present, this heterogeneity is not reflected in the packaging process.

Viability of uracil-containing T4 phage

The viability of these phage in *ung*⁺ and *ung*⁻ hosts is shown in Fig. 1. The T4-U phage replicate as well in *ung*⁻ cells as do T4-T

Table 2 Comparison of total intracellular DNA with packaged phage DNA

	H	% Radioactivity C	T	U
Intracellular DNA	9	27	41	23
Packaged DNA	8	26	41	25

E. coli BW212 was infected with mutant T4 in the presence of [³H]uridine as described in Table 1. The total DNA was isolated from one portion of the infected culture and its base composition was determined¹⁸. The mature phage were isolated from the remainder of the culture by CsCl gradient centrifugation, and the base composition of the phage DNA was also determined.

phage in either *ung*⁺ or *ung*⁻ cells (Fig. 1a). In contrast, the T4 U phage fail to replicate in *ung*⁺ cells, producing an average of less than one progeny phage per infected cell by 75 min after infection; this is about 0.2% of the burst observed in *ung*⁻ cells. Thus, uracil-containing phage are viable in *ung*⁻ *E. coli* cells, but are restricted by wild-type *E. coli*.

It is possible that transcription of uracil-containing DNA in *ung*⁻ cells might be impaired, but that the presence of more than one copy of each gene in the cell permits rescue of impaired functions by either recombination or high gene dosage. To rule this out, cultures were infected at a multiplicity of infection of less than one (Fig. 1b). In this experiment, the T4-U phage replicated essentially as well as T4-T phage, indicating that there is at most a very slight impairment of either transcription or DNA replication.

Metabolism of uracil-containing T4 DNA

The above results show that the presence of uracil-DNA glycosidase in *E. coli* severely restricts the replication of T4 phage containing as much as 30% of its thymine replaced by uracil. This suggests that the parental uracil-containing DNA is subject to attack by the glycosidase and that in the process it is damaged beyond repair. The results shown in Fig. 2 confirm that up to 60% of the parental uracil-containing T4 DNA is very rapidly degraded to acid-soluble form within the first 2 min after infection, and that this degradation does not take place in *ung*⁻ cells or when T4-T phage are used. Little further acid-solubilisation occurs after the first 2 min, and if the incubation is continued for as long as 15 min some of the acid-soluble radioactivity is reincorporated into RNA and DNA (data not shown).

Killing of *E. coli* by uracil-containing T4 phage

The very rapid degradation of the uracil-containing DNA in *ung*⁺ cells suggests the possibility that the infecting DNA may be destroyed before it can even be transcribed. If so, some cells might recover and survive the infection as happens after ghost infection¹⁴. We have observed that more than 10 times as many *ung*⁺ cells survive infection with T4-U phage as survive infection with T4-T phage (Fig. 3a). The data even suggest that some cells regain viability if the incubation is continued for up to 15 min in liquid culture. When *ung*⁻ cells are infected, they are killed equally well by T4-T and T4-U phage (Fig. 3b). The results cannot be explained by poor adsorption of T4-U phage since T4-U and T4-T phage adsorb at similar rates to *E. coli* W3110 (Fig. 3c). From these results we concluded that *ung*⁺ cells infected by uracil-containing T4 phage do survive the infection 10–20% of the time because of rapid destruction of the parental phage DNA. The survival might even be greater if cells were infected by fewer phage, since the multiplicity of infection used in the experiments shown in Fig. 3a and b were mostly in the range 5–10. The results obtained with T4-U phage are qualitatively similar to those obtained using ghosts prepared from wild-type phage¹⁴.

Discussion

In the experiments described here about 30% of the thymine was replaced by uracil during synthesis of T4 DNA. These DNA molecules apparently function reasonably well, showing that the

presence of this much uracil in the DNA can be tolerated by the various proteins which must interact with the DNA during replication and transcription. We have not yet determined whether 100% replacement of thymine by uracil can be equally well tolerated. Also unanswered is the question of how much uracil can be tolerated before the ability to repair the DNA is overwhelmed by the degradative process associated with the base excision repair pathway. Clearly, when 30% of the pyrimidines are uracil the DNA is degraded too rapidly for repair to keep pace.

We have observed some acid-solubilisation of the uracil-containing DNA in the *ung*⁻ host. This may be due to residual N-glycosidase activity *in vivo*, although we cannot detect activity *in vitro* (in preparation). Alternatively, it may be due to uracil-specific degradation by the endonuclease described by Gates and Linn¹⁵. Our data do suggest that this latter enzyme has only a minor role, if any, during repair of uracil-containing regions of DNA in *E. coli*.

In our experiments the uracil-DNA glycosidase is presumably attacking A·U base pairs in native DNA. Although Lindahl *et al.*¹⁶ have shown that *in vitro* the enzyme may have a slight preference for G·U mismatches over A·U base pairs, the enzyme does not possess sufficient specificity to selectively repair G·U mismatches in DNA containing A·U base pairs rather than A·T base pairs. Since the frequent random substitution of uracil for thymine during T4 DNA synthesis seems to have no serious consequences for the subsequent replication of these T4 phage, the occasional misincorporation of dUTP in place of dTTP into *E. coli* DNA may have only minor effects on replication and transcription of *E. coli* DNA. If so, the most critical role of uracil-DNA glycosidase *in vivo* may be to prevent transition mutations caused by deamination of cytosine to uracil in DNA¹⁶;

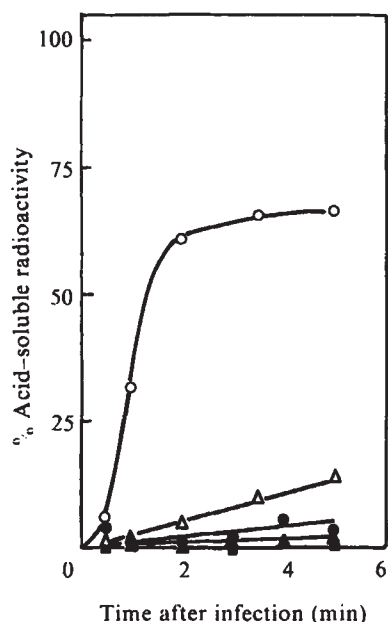


Fig. 2 Acid-solubilisation of parental phage DNA. *E. coli* W3110 and BD10 were infected with wild type and T4·U or T4·T phage at low multiplicities (0.4–0.7) to maximise the amount of parental phage DNA injected. These phage had been synthesised in the presence of [6-³H]uridine and the distributions of radioactivity in the phage were comparable to those shown in Table 1. At various times samples were removed and the nucleic acids were precipitated with 0.1 volume of cold 50% trichloroacetic acid. These suspensions were centrifuged, and the amount of radioactivity present in the supernatant solutions was determined by counting portions using Triton X-100: toluene (1:2) containing 0.6% PPO as the scintillation fluid. The results shown above correspond to phage with no cells present (■), wild type T4 with *E. coli* W3110 (▲), T4·T with *E. coli* W3110 (●), and T4·U with *E. coli* BD10 (△), and W3110 (○). The precipitates were also washed twice with cold 5% trichloroacetic acid dissolved in 0.3 M NaOH, and portions were counted. The results are not shown, but the decreases in acid-insoluble radioactivity corresponded well with the increases in acid-soluble radioactivity shown above.

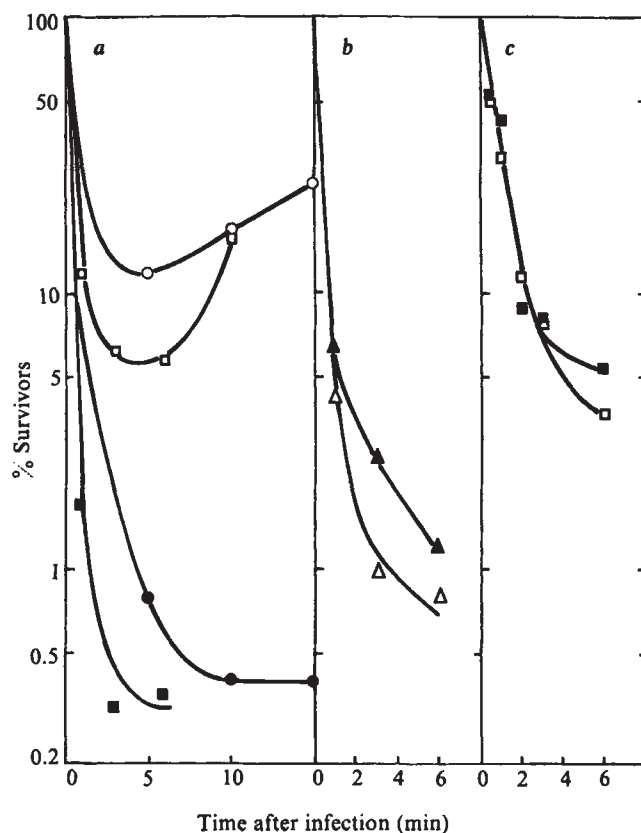


Fig. 3 Killing of cells infected with uracil-containing phage. *E. coli* W3110 was infected with mutant phage at high multiplicity in two separate experiments, and the number of surviving cells was measured at various times (a). The multiplicities of infection were 9 and 5 for T4·T (●, ■) and 11 and 4 for T4·U (○, □). When *E. coli* BD10 was infected (b), the multiplicities of infection were 5 and 6 for T4·T (▲) and T4·U (△), respectively. In (c) the unadsorbed phage were measured after treating portions of the infected W3110 with chloroform. The multiplicities of infection were 0.7 and 0.9 for T4·T (■) and T4·U (□) phage, respectively.

the presence of A·T base pairs in DNA rather than A·U base pairs permits specific recognition and repair of potentially mutagenic G·U mismatches produced by cytosine deamination. It is not yet clear whether frequent substitution of uracil for thymine in *E. coli* DNA has serious consequences for replication and expression of *E. coli* DNA, although *E. coli* in which about 10% of the thymine has been replaced by uracil do grow at about one-half the rate of normal *E. coli* (data not shown).

This work was supported by US PHS grant GM 21464 to H.R.W. and Postdoctoral Fellowship GM 05401 from the National Institute of General Medical Sciences to B.K.D. We thank Elizabeth Kutter and Larry Snyder for the gift of the *alc* mutant T4 phage, and Bernard Weiss for constructing strain *E. coli* BW212 (*dut-11 ung-1*), and for useful discussions.

Received 28 November 1977; accepted 9 January 1978.

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letters to nature

On falling through a black hole into another universe

AMONG the exact solutions to Einstein's field equations of general relativity are those describing black holes which rotate and/or carry electric charge. These solutions are novel in that they may be analytically continued through the black hole interior in a time-like direction into a succession of asymptotically flat spacetime regions that are inaccessible in the spacetime region in which the black hole first formed. There has been speculation about whether matter could travel through such black holes into these 'other universes'. As real black holes will never be precisely non-rotating and electrically neutral, it is of interest to determine whether such a transfer of matter from one universe to another is possible. If it were, it might imply the possibility of matter appearing explosively in our region of spacetime through white holes. The exact solutions of interest are suspect because they are idealised. For example, they exclude the effects of matter surrounding the hole, and quantum processes. It has been suggested^{1,2} that the interior of the idealised black hole might be smashed in a more realistic model by unbounded blue shift effects associated with classical matter falling along the so-called inner horizon. In this way the space bridge would be destroyed by back reaction of the gravitational field due to the energetic matter. Here we demonstrate that quantum vacuum effects, similar in origin to the attraction energy between two electrically neutral conducting plates (Casimir effect), also cause an unbounded back-reaction which would smash the idealised interior geometry, even if no actual matter were falling into the hole. We do not speculate whether other analytically extendible spacetimes exist which these quantum processes leave topologically unmodified, but demonstrate that the models which have been explicitly advanced to date cannot be taken seriously as space bridges.

The black hole solutions which contain the feature under discussion have the causal structure indicated by the conformal Penrose diagram, Fig. 1. We restrict discussion to the Reissner-Nordstrom solution (non-rotating, electrically charged black hole) because its spherical symmetry simplifies the calculation. However, the result depends on the causal, rather than geometrical structure of the spacetime, and we see no reason why the analysis would not extend to the rotating case.

The radial coordinate is denoted by r . The usual singularities at $r = 0$ are time-like, and this allows the spacetime to be continued through a bridge between the singularities, and out into another universe. The surface $r = r_+ = M + (M^2 - e^2)^{1/2}$ is the outer horizon, which forms the boundary of the causal past of $\mathcal{J}^+$ in our Universe and is a surface of infinite red shift. Our attention centres on the other, inner horizon, $r = r_- = M - (M^2 - e^2)^{1/2}$, which is a surface of infinite blue shift for ingoing null rays. The metric of the spacetime is

$$ds^2 = -\left(1 - \frac{2M}{r} + \frac{e^2}{r^2}\right)dt^2 + \left(1 - \frac{2M}{r} + \frac{e^2}{r^2}\right)^{-1}dr^2 + r^2(d\theta^2 + \sin^2\theta d\phi^2) \quad (1)$$

We consider the modification of this spacetime by the presence of a massless, electrically neutral, quantum field, which in practice would be, for example, the electromagnetic or neutrino field. To a first approximation, we consider the background geometry to be

fixed, and calculate the stress-energy-momentum tensor, $T_{\mu\nu}$, of the quantum field on this background. We shall show that even when there is no field energy falling into the black hole, one component of the stress tensor, evaluated in coordinates which remain smooth, still diverges along $r = r_-$, through vacuum polarisation effects.

There is still some controversy surrounding calculations of quantum field theory in curved spacetime, partly because the theory is only a semi-classical approximation. Fortunately, however, our result does not depend on detailed calculations from the theory of quantum fields, but can be extracted merely from the covariant conservation equation

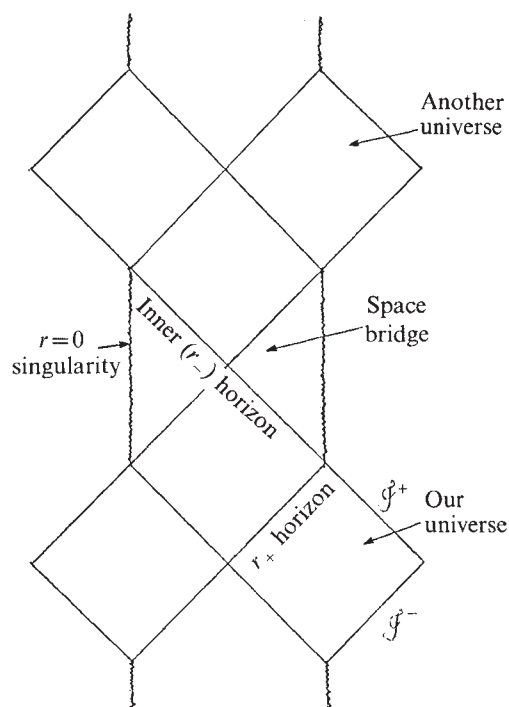
$$T^{\mu\nu}_{;\nu} = 0 \quad (2)$$

subject to spherical symmetry, time independence and the known form of $T_{\mu\nu}$ at spatial infinity where the spacetime is in any case flat. In particular, the only regularisation required is in the assumption of the so-called anomalous trace T^μ_μ . This trace has been calculated in several ways³⁻⁹. Moreover, in two dimensions its absence would imply that there is no particle production³. Thus, given the framework of this semiclassical theory we do not make any additional very strong assumptions. We follow the discussion already given by Christensen and Fulling¹⁰ for the Schwarzschild black hole. We work in null coordinates

$$u = t - r^*, v = t + r^*, r^* = \int \frac{dr}{(1 - 2M/r + e^2/r^2)} \quad (3)$$

The chosen quantum state must be consistent with the assumed topology which is time-symmetric. Thus $T_{\mu\nu}$ will be a function of r

Fig. 1 Conformal diagram showing the spacetime associated with an electrically charged black hole analytically extended through the black hole (space bridge) into other universes.



only. We also require that $T_{\mu\nu}$ remain regular at the outer horizon $r = r_+$, to avoid blocking off the hole completely. Finally, we choose the quantum state to be a vacuum state of the field corresponding to no matter falling through into the hole ($T_{\nu\nu} = 0$ at $r = r_+$). Relaxing this assumption would only make the effect we predict more pronounced.

The essential qualitative features of interest are present in the two-dimensional model spacetime in which the angular coordinates in equation (1) are discarded. It turns out that the three conditions above already uniquely determine $T_{\mu\nu}$, once the anomalous trace T_α^α is supplied:

$$T_\alpha^\alpha = \frac{R}{24\pi} = \frac{M}{6\pi r^3} \left(1 - \frac{3e^2}{2Mr}\right) \quad (4)$$

A simple integration of equation (2) then yields for the ingoing null flux

$$T_{\nu\nu} = \frac{1}{24\pi} \left[\frac{M^2 - e^2}{2r_+^4} - \frac{M}{r^3} + \frac{3(M^2 + e^2)}{2r^4} - \frac{3Me^2}{r^5} + \frac{e^4}{r^6} \right] \quad (5)$$

which vanishes at $r = r_+$. At infinity $T_{\nu\nu} = T_{uu}$, so T_{rr} , the net radial flux, vanishes as required by the overall time symmetry. The energy density there, $T_{uu} + T_{\nu\nu}$, does not vanish. This energy is, in fact, the Hawking thermal radiation in thermodynamic equilibrium with the black hole¹¹. (We could not relax the equilibrium and allow the black hole to evaporate, because it would no longer have the topology shown in Fig. 1. Whether an evaporating, charged black hole would have some other topology involving analytic continuation to another asymptotically flat spacetime region is, of course, unknown.)

To examine the physical situation represented by $T_{\nu\nu}$ at $r = r_-$, we must transform to a coordinate system that is non-singular along this null surface. One such transformation is

$$V = \tan^{-1} \left[\exp \left(\frac{r_+ - r_-}{2nr_-^2 v} \right) \right] \quad (6)$$

where n is an integer $\geq 2r_+^2/r_-^2$. Thus

$$T_{\nu\nu} = \left(\frac{nr_-^2}{r_+ - r_-} \right)^2 \frac{1}{\sin^2 2V} T_{\nu\nu} \quad (7)$$

The horizon $r = r_-$ is represented by the null surface $V = \pi/2$ in these coordinates. Clearly $T_{\nu\nu}$ will become singular at r_- unless $T_{\nu\nu}$ vanishes there. Substituting $r = r_-$ into equation (5) yields

$$T_{\nu\nu}(r = r_-) = \frac{(M^2 - e^2)}{48\pi} \left(\frac{1}{r_+^4} - \frac{1}{r_-^4} \right) \quad (8)$$

which is non-zero. Hence $T_{\nu\nu}$ is singular on the inner horizon. This result has also been reported by Hiscock¹² in a slightly different context.

In the more realistic four-dimensional case, using the full metric¹, it is not possible to evaluate $T_{\mu\nu}$ directly by regularisation of mode sums in terms of known functions. In two dimensions the spacetime is conformally flat, enabling simple exponential mode solutions to be used, but in four dimensions the spacetime is not conformally flat. Physically this implies that radiation can be backscattered off the spacetime curvature, which means that an outgoing energy flux reflects back into the black hole and vice versa. The effect is very complicated and manifests itself in the radial wave modes, which have to be handled numerically or approximately^{10,13}. Fortunately, it is not necessary to know the exact form of $T_{\mu\nu}$ to investigate whether it remains finite at the inner horizon.

Following along the lines of the treatment for the two dimensional case, we integrate the conservation equation (2), subject to spherical symmetry, time-independence, finitude on r_+ and zero net flux at large r . The result is

$$T_{\nu\nu} = \frac{1}{2r^2} [G(r) + H(r)] + \frac{1}{2} \left(1 - \frac{2M}{r} + \frac{e^2}{r^2} \right) [\theta(r) - \frac{1}{4} T_\alpha^\alpha(r)] \quad (9)$$

where

$$\theta(r) \equiv T_\theta^\theta(r) - \frac{1}{4} T_\alpha^\alpha(r) \quad (10)$$

$$H(r) \equiv \frac{1}{2} \int_{r_+}^r (\bar{r} - M) T_\alpha^\alpha(\bar{r}) d\bar{r} \quad (11)$$

$$\text{and} \quad G(r) \equiv 2 \int_{r_+}^r \left(\bar{r} - 3M + \frac{2e^2}{\bar{r}} \right) \theta(\bar{r}) d\bar{r} \quad (12)$$

Once again, the anomalous trace T_α^α is known. In this spacetime it is

$$(1440\pi^2)^{-1} \left[+ \frac{53e^4}{r^8} - \frac{84e^2M}{r^7} + \frac{42M^2}{r^6} \right] \quad \text{for neutrinos} \quad (13)$$

$$(360\pi^2)^{-1} \left[- \frac{47e^4}{r^8} + \frac{156e^2M}{r^7} - \frac{78M^2}{r^6} \right] \quad \text{for photons}$$

Unlike two dimensions, $T_{\nu\nu}$ is not completely determined by the trace; there is the unknown angular component T_θ^θ , the evaluation of which requires the integration of the radial mode sum. T_θ^θ will be a complicated function of e , M and r which cannot be written down, like the trace, in terms of simple inverse powers of r .

In order for the $v-v$ component of $T_{\mu\nu}$ to remain finite in the coordinate system equation (6), which is regular at $r = r_-$, $T_{\nu\nu}$ must approach zero as $r \rightarrow r_-$. From equation (9) this requires

$$G(r_-) + H(r_-) = 0 \quad (14)$$

$H(r_-)$ may be evaluated exactly using equations (11) and (12). The result is, for photons plus neutrinos

$$H(r_-) = \frac{1}{2880\pi^2} \left[\frac{45e^4}{2r_-^6} - \frac{135e^4M}{7r_-^7} - 108 \frac{e^2M}{r_-^5} + 90 \frac{e^2M^2}{r_-^6} + \frac{135M^2}{2r_-^4} - 54 \frac{M^3}{r_-^5} \right]_{r_+}^{r_-} \quad (15)$$

which is non-zero (except perhaps on a set of measure zero in e space, corresponding to roots of equation (15)).

Unfortunately, it is not possible to evaluate $G(r_-)$ using known functions, because of the complexity of the radial modes which enter into T_θ^θ , which will be a complicated function of e , M and r . However, because of equation (15), the left hand side of equation (14) is non-zero, except under the improbable circumstances that the complicated mode-dependent integral (12) exactly cancels the simple polynomial equation (15) on some open set of e , M values. Even if this were to occur, it would not be of interest in the real universe because the functions T_θ^θ and $G(r)$ depend on the choice

of field modes, and hence the quantum state. It is a non-local, non-geometrical quantity. However, T_α^2 , which determines equation (15), is a local, geometrical scalar, and is independent of the quantum state. Thus, any slight perturbation about the precise thermodynamic equilibrium state chosen here, which obviously will occur in the real universe, will alter $G(r_-)$ but not $H(r_-)$, and destroy the exact cancellation. Similar remarks apply to the existence of any roots of equation (15).

These general conclusions are unchanged if, instead of treating the maximally extended spacetime, we deal with a black hole formed by an imploding object. In two dimensions T_{vv} cannot be affected on causality grounds (this was the case treated by Hiscock¹²). In four dimensions, backscattering of radiation emitted by the collapsing matter will occur, and this can contribute to T_{vv} along r_- . However, it will depend sensitively on the details of the collapse, which are in general very complicated. (It is only in the region just outside r_+ that $T_{\mu\nu}$ is independent of the collapse details. This was the situation considered by Hawking¹¹.) So again, exact cancellation of T_{vv} , even if it should be contrived for some particular model of the collapse, would not occur in the real universe, because these different contributions which go to make up T_{vv} depend on different physical circumstances for their values.

In practice, back-reaction of the quantum fields would become important near r_- , with the result that the interior of the Reissner-Nordstrom black hole would be smashed. Consequently, this model of a charged black hole with a space bridge to other universes is an unrealistic idealisation.

N.D.B. was supported by a Flinders University Overseas Scholarship. We thank W. A. Hiscock for valuable comments.

N. D. BIRRELL

P. C. W. DAVIES

Department of Mathematics,
King's College,
Strand,
London WC2, UK

Received 26 October 1977; accepted 10 January 1978.

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Hard X-ray observations of white dwarf binary systems

THE ability of degenerate dwarfs, as opposed to neutron stars, to radiate at X-ray wavelengths has created much theoretical interest. Ariel 5 hard X-ray observations indicate that current theories seem inadequate to explain all aspects of the observations. Using the Imperial College hard X-ray scintillation telescope (ST) on Ariel 5, several short period (up to 12 h) binary star systems have been studied and are described here. This study was carried out after a very hard X-ray flux was detected from AM Herculis leading to a search for similar signals from optically similar star systems. The detector used consists of 8 cm² of CsI crystal actively collimated to 8° FWHM and covers the energy range 26–1,200 keV. It is offset from the satellite spin axis, thereby enabling background subtraction using a modulation technique. The instrument and its operation are described fully elsewhere¹.

A search for hard X-ray emission from DQ Herculis was carried out during 18–22 February 1977. This white dwarf binary system has been proposed as a possible hard X-ray source by Fabian *et al.*² and Katz³. Detailed study of the signal that was detected revealed that it was inconsistent with the position of DQ Herculis but fitted AM Herculis. The fit to a source positioned at AM Herculis' position was, in fact, sufficiently good to enable upper limits on any flux from DQ Her to be set. The results are shown in Fig. 1, together with data from the Ariel 5 Sky Survey Instrument⁴ and the OSO-8 proportional counter experiment⁵. The best fit to the data is a power law with exponent (−0.84). Any thermal-type spectrum is inconsistent with the observations as a whole and can only be used to explain part of the data (for example, thermal bremsstrahlung with $kT \geq 400$ keV could fit the high-energy tail, but still not as well as the power law). The net probability that the ST results are due to counting statistics is 1.1×10^{-6} . Also shown are the two standard deviation upper limits for DQ Herculis for three energy bins assuming a similar spectral form. The lowest energy bin comes from the Ariel 5 Sky Survey Instrument (B. Cooke, personal communication).

Due to poor time resolution in the mode of observation used (> 512 s) no search was carried out for spin periods such as those seen in the optical emission of DQ Herculis⁶. However, a search through the data for modulation with the binary periods of AM Her or DQ Her was carried out using periods published Swank *et al.*⁵ and Nelson⁶, respectively. It was hoped that this would help in distinguishing the sources, but no very conclusive results were obtained. Slightly stronger modulation was found with the period of AM Her, enabling a two standard deviations upper limit at 76% to be set for any modulation in the energy interval 260–1,200 keV in phase with the soft X rays⁵.

A search was then made through the Ariel 5 hard X-ray data for other possible short-period binaries that may emit X rays.

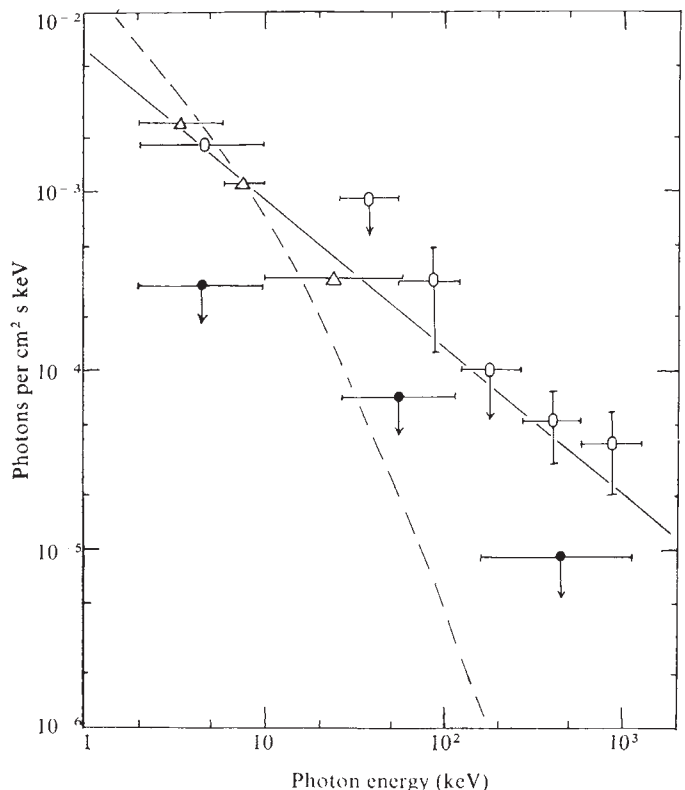


Fig. 1 Spectral observations of AM Herculis and DQ Herculis showing (○) Ariel 5 data on AM Her; (△) OSO-8 data on AM Her; and (●) Ariel 5 two standard deviations upper limits on DQ Her. The solid line represents the power law fit to all the AM Her data, and the dashed line represents the thermal prediction of Katz³.

Four such candidates were studied, VV Puppis, V Sagittae, AM Canum Venaticorum and EX Hydrae, but only AM Canum Venaticorum showed any indication of hard X-ray emission. This source gave a 2.5 standard deviation signal in the 260–1,200 keV band. Combining the probability of this deviation with the probability of the signal phase also being correct, brings the total chance of this detection being due to counting statistics to 2.5×10^{-3} . A summary of all these observations is presented in Table 1. Flux upper limits are two standard deviations.

This group of sources are all cataclysmic variables and the optical properties of three of them have been discussed by Warner⁷. They all incorporate white dwarfs in their binary systems, and at least three of them (DQ Her, AM Her and VV Puppis) show strong optical polarisation indicative of intense magnetic fields. In addition, spin periods have been optically detected in both DQ Her⁶ and V Sagittae⁸. It might, therefore, be reasonable to look for a spin periodicity in the X-ray emission of AM Herculis.

Table 1 Ariel 5 hard X-ray observations

Source	Position α δ	Binary period (min)	Flux in 260–1,200 keV band (Photons per cm ² s keV $\times 10^{-5}$)	Observa- tion date
AM Herculis	273.3 +50.0	186	5.0 ± 1.7	Feb 1977
DQ Herculis	271.5 +45.9	279	≤ 0.96	Feb 1977
VV Puppis	122.6 -18.8	100	≤ 2.64	Oct 1975
V Sagittae	304.0 +20.8	740	≤ 1.74	June 1975
AM Canum Vena- ticorum	187.5 +38.2	18	1.74 ± 0.7	Dec 1976
EX Hydrae	191.7 -28.7	98	≤ 1.77	Dec 1975

If a distance of 100 pc is assumed for AM Her, then an integrated X-ray luminosity from 1 keV to 1 MeV can be calculated to be 5×10^{32} erg s⁻¹. Assuming a similar spectral form for DQ Her and a distance of 250 pc, then a two standard deviations upper limit of 2.6×10^{33} erg s⁻¹ is found. The later figure is somewhat less than the flux of 10^{34} erg s⁻¹ estimated by Fabian *et al.*².

Katz³ has made detailed spectral predictions for optically thin, thermal bremsstrahlung emission arising from spherical accretion on to a degenerate dwarf. He considers both the case of a shock front created close to the dwarf and one far away, and his prediction for the former, normalised to our estimated AM Her luminosity, is shown in Fig. 1. The other case produces a curve very similar in shape but at a slightly lower predicted intensity. Both seem to fail to explain the flat power law shape and X-ray intensities above 100 keV. If we accept that a degenerate dwarf, AM Her, is responsible for the observed spectrum, then thermal bremsstrahlung from a distant shock in the slow-settling regime of Katz does not seem to provide an adequate explanation.

These systems, in general, are all short period variables, and it is interesting that the one with the shortest period, AM Canum Venaticorum, shows evidence of being an X-ray emitter. Also worth noting is that the star EX Hydrae additionally belongs to the U Geminorum class, the original of which has recently been discovered by HEAO-1 to be a soft X-ray emitter⁹, and it lies just outside the error boxes for 4U1249-28 and 2A1251-290.

Finally, there are three other sources in this class noted by Warner⁷. VW Hydri, WZ Sagittae and Z Chamaeleontis, but they have not been looked at by the Ariel 5 ST experiment. These, together with the other sources discussed here, should be worth further investigation.

We thank C. Sowden for computing assistance and the spacecraft management by the Appleton Laboratory.

M. J. COE

A. R. ENGEL

J. J. QUENBY

The Blackett Laboratory,
Imperial College,
London SW7, UK

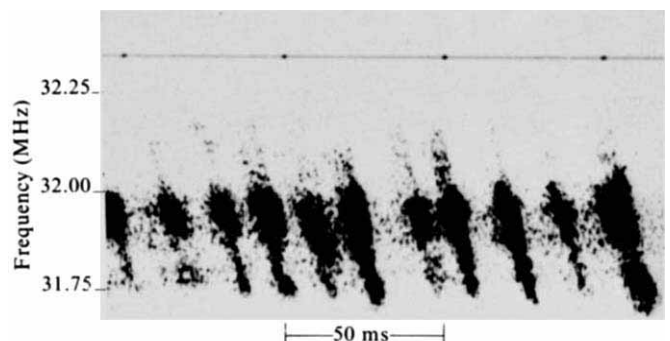
Received 6 December; accepted 22 December 1977.

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Jovian S-burst observations at 32 MHz

JUPITER emits highly sporadic and intense radio emission in the 0.45–40 MHz frequency range¹. Several high-resolution observations below 28 MHz have revealed that a significant fraction of this radiation, perhaps 25%, is comprised of S bursts, which are characterised by rapid negative drift rates R (tens of MHz s⁻¹) and extremely narrow instantaneous bandwidths (≤ 200 kHz²⁻⁷). The probability of observing S bursts, which are associated exclusively with source regions B and C, is determined to a large extent by the geocentric phase of Io. This has led to the widely-held conclusion that the emission is beamed from the planet's Io-associated magnetic flux tube (IFT). S-burst emission models generally assume simple adiabatic motion of trapped electrons accelerated at Io and radiating at or close to the local electron gyrofrequency (refs 8–10). The details of the emission process are unclear and at present offer no restrictions as to the sign of R , however, as the S bursts exhibit negative frequency drift ($R < 0$), we shall speak only in terms of electron bunches radiating as they stream outward from their mirror point near the IFT foot. In this context $|R|$ is predicted to increase rapidly from zero to some maximum value (depending upon electron energy) as the particles execute this motion. In terms of frequency, this region of the northern hemisphere IFT corresponds to $f_s \gtrsim 27$ MHz. A determination of R at several points in this frequency range is then a critical test of models incorporating a trapped-electron hypothesis. Accordingly, we present here a preliminary report on observations conducted in this range.

Fig. 1 Segment of 32-MHz S burst storm of 8 August 1976. Mean drift rate $R_m = -29.6$ MHz s⁻¹, mean instantaneous bandwidth = 171 kHz.



The S-burst activity was recorded at the Maipo Radio-astronomical Observatory of the University of Chile on 8 August 1976 from 1100–1104 UT at a frequency of 32 MHz. The System III (1965) longitude and geocentric Io phase at the time of emission were $\lambda_{\text{III}} = 144^\circ$ and $\lambda_{\text{Io}} = 88^\circ$ respectively, corresponding to the Io–B source. The receiving equipment consisted of a linearly polarised, 15 full-wave dipole array, a 500-kHz bandwidth receiver, and a high-speed instrumentation tape recorder. Subsequent data processing using the University of Florida variable time-expansion spectrograph yielded frequency and time resolutions of 3.2 kHz and 300 μs across the 500 kHz data band. Burst spectra were recorded on film for visual analysis and display⁶.

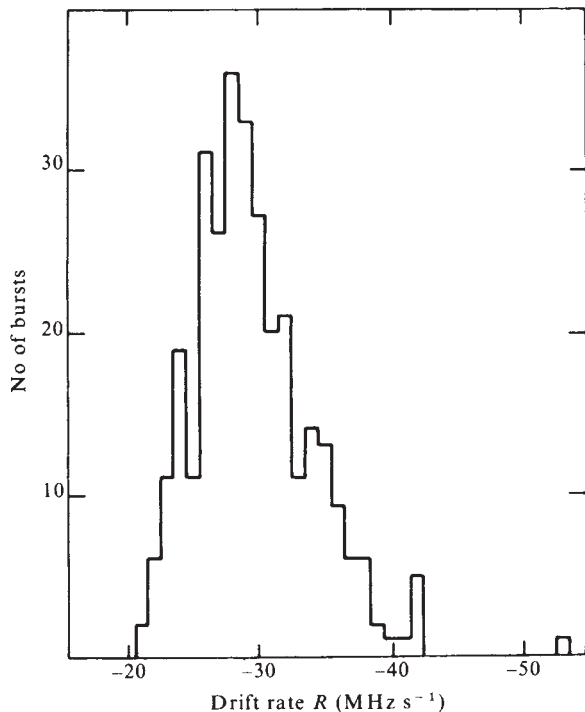


Fig. 2 Drift-rate distribution of 312 S-bursts. $R_m = -29.6 \pm 4.6$ (1 σ) MHz s⁻¹, 32-MHz S-burst storm, 1100–1104 UT, 8 August 1976.

A typical segment of the data film is shown in Fig. 1. Each S-burst drifts in a negative sense, from high to low frequency, and exhibits a general uniformity in overall structure. The drift rates are not constant from burst to burst but vary over a considerable range of values. The actual distribution, based upon measurement of 312 bursts, is shown in Fig. 2. As the uncertainty of individual measurements is small, < 0.25 MHz s⁻¹, random processing errors do not contribute in any way to the observed spread in drift rates. It is clear that the distribution is not symmetrical but displays positive skew, a feature held in common with observations at lower frequencies⁷. The peak and the arithmetic mean of the sample occur at drift rates $R_p = -28$ MHz s⁻¹ and $R_m = -29.6 \pm 4.6$ (1 σ) MHz s⁻¹, respectively. Contrary to expectations, we find no evidence for bursts with R near zero; in fact, the sample mean R_m is among the largest of any recorded at lower frequencies.

In order to place the present results in context, we show (Fig. 3) R_m values of all available Io–B S-burst observations. Several theoretical drift rate curves, obtained by assuming adiabatic electron motion on the Northern-Hemisphere IFT, are included for discussion. (We ignore the Southern-Hemisphere IFT because $f_g \leq 25$ MHz there.) Although different $\lambda_{\text{III}}-\gamma_{\text{Io}}$ coordinates apply to each of the observations shown, we use a nominal Io–B source geometry (λ_{III} (1965) = 140° , $\gamma_{\text{Io}} = 90^\circ$)

in designating B_{IFT} . This is accurate enough as the theoretical curves are relatively independent of variations in source geometry. The vector magnitude and direction of B_{IFT} , necessary for implementing the curves, are derived from a Pioneer 10–11 magnetic field model¹¹. In these conditions, f_g at the northern IFT foot is 34.8 MHz, as indicated by the dotted line in Fig. 3.

In generating each curve we have chosen a different electron energy and equatorial pitch angle (explicitly noted), thus securing a fit to three different observational regimes. Curve *a* is representative of predictions based on models employing mirroring electrons; it is seen to match the low $|R|$, lower-frequency observations extremely well. As curve *b* indicates, however, acceptable agreement with the 32 MHz results and the high $|R|$ values evident at several lower frequencies cannot be achieved except by using electrons that are well within the atmosphere loss cone. We emphasise this point through curve *c*, which does use mirroring electrons but which fails to match any but the 32 MHz value. It is evident then that, unless each observation is considered independent of the rest, a substantial fraction of the observations cannot be accounted for by trapped electrons accelerated at Io. We propose that the particles may therefore have been accelerated initially in the Jovian ionosphere at the IFT foot. Conjectures regarding the existence of both Jovian^{8,12} and terrestrial¹³ ionospheric acceleration mechanisms have been made previously.

In this context, the burst spectra illustrate qualitatively the behaviour of the electron bunches shortly after injection into the IFT. Considering this view, we note that the lower-frequency spectra (5–28 MHz) reveal a much greater variety of burst forms than the relatively uniform burst structure apparent in Fig. 1^{3,6}.

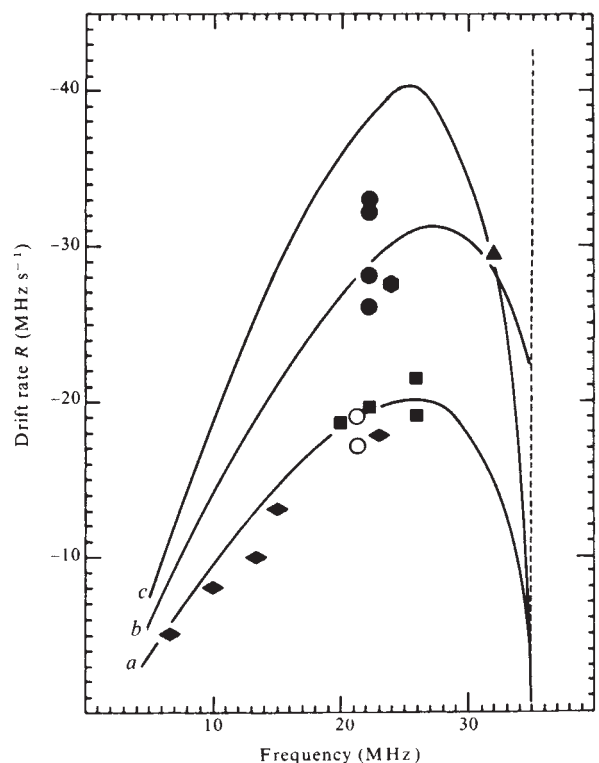


Fig. 3 Three theoretical drift-rate curves are compared with all Io–B S-burst storms for which drift rates have been measured (several of Ellis's observations have been averaged here). The dotted line indicates f_g (34.8 MHz) at the Northern-Hemisphere IFT foot; use of non-mirroring electrons in case *b* (6 keV, 2.24°) yields a finite value for R at this point. In cases *a* (3 keV, 2.32°) and *c* (12 keV, 2.32°), electrons are used which mirror at the IFT foot. The observations are well-mixed in time ($\blacktriangle$, present work; $\blacklozenge$, 1975 data³; $\circ$, 1974 data¹; $\bullet$, 1976 data⁷; $\blacklozenge$, 1973 data⁵; $\blacksquare$, 1971 to 1974 data⁶), thus the broad range of observed values near 22 MHz probably does not reflect a secular change in the system responsible for the bursts.

Specifically, highly unusual spectral behaviour has been reported between 5 and 18 MHz, including herringbone structure, U bursts, and a few instances of positively drifting bursts³. Although it may be premature to generalise in regard to a frequency-dependent morphology, it seems that structural complexity increases with decreasing frequency. This may be understood in terms of electrons entering the IFT in a uniform manner and subsequently exhibiting the cumulative effects of velocity and density modulation as they stream outward¹⁴. This scenario thus lends qualitative support to the hypothesis of an ionospheric acceleration mechanism.

The extent to which this conclusion can be substantiated will depend largely upon two factors: the results of further observations in the high-frequency regime, and refinements in the near-surface magnetic field models to follow from the Voyager spacecraft measurements.

This work was supported in part by grants from the US NSF and NASA. We thank Francisco Reyes, Eugenio Covacevich and Eduardo Diaz for collaborating with this project.

MICHAEL D. DESCH
RICHARD S. FLAGG

Department of Physics and Astronomy,
University of Florida,
Gainesville, Florida 32611

JORGE MAY

Maipu Radioastronomical Observatory,
University of Chile,
Maipu, Chile

Received 26 October; accepted 16 December 1977.

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Jupiter's S bursts and Io

THE correlation between the occurrence of the Jupiter radio bursts and the position of the satellite Io has been reported to exist mainly for wave frequencies between 20 MHz and 40 MHz (ref. 1). Below about 17 MHz little correlation has been observed². Jupiter's radio bursts may be roughly divided into two classes: the long, or L, bursts with a single frequency duration of seconds and the short, or S, bursts with a single frequency duration of 1–10 ms. Previous investigations of the Io effect have not distinguished between these two types of bursts. Here we describe a separate study on S bursts in which their occurrence probability is found to be strongly correlated with the position of Io above and below 17 MHz. In order to detect this result, spectrum analysers capable of separating out the S bursts from other jovian emission over a large frequency range had to be used³.

From 1972 to 1974 the Llanherne low frequency radio telescope⁴ was used to monitor Jupiter's emission in the 2–17 MHz frequency range⁵. In 1975 a much smaller telescope was used to gather data on Jupiter's emission in the 11–24 MHz frequency range⁶. On each observing day, the signals were recorded on six videotape recorders for 5 min near transit and subsequently

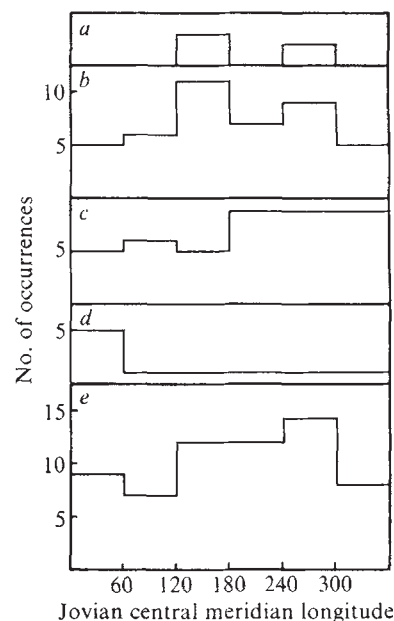


Fig. 1 Number of occurrences of S bursts against jovian meridian longitude in the frequency ranges: a, 21–24 MHz; b, 14–17 MHz; c, 11–14 MHz; d, 8–11 MHz; e, total.

analysed with a 256-channel spectrum analyser with frequency resolution of 10 kHz and time resolution of 600 μ s. During 1972–74 jovian emission was detected in 145 transits out of 283 observed. Of those transits where emission was detected, S bursts were observed on 53 occasions. Thus S bursts were observed in more than a third of the transits where Jupiter emission was detected. In 1975, S bursts were seen nine more times. Analysis of the 62 S burst events has revealed that throughout the frequency range 8–24 MHz, the probability of observing S bursts is greatly enhanced when Io is near 90° or 230° from superior geocentric conjunction (SGC). In addition, S bursts were observed below 17 MHz at all central meridian longitudes (CML) of Jupiter in the present observations, contrary to previous reports that they are confined to a narrow range of CML in this frequency range⁷.

Figure 1 shows histograms of S burst occurrence against jovian CML for each of the observing frequency ranges. The new System III (1965) (ref. 8) for longitudes has been used. Apart from the 21–24 MHz observations, S bursts in the 8–11, 11–14 and 14–17 MHz frequency ranges were observed at all jovian longitudes. However, a histogram of S burst occurrence against Io's deviation from SGC (Fig. 2) reveals a very strong correlation at all observed frequencies. No such correlation has been found for the longer-duration slowly drifting L bursts or other less frequently occurring classes of millisecond bursts⁵.

Jupiter's S bursts have narrow bandwidths, which may be as small as 2 kHz, and are sporadic and very intense. They exhibit a rapid negative frequency drift of the order of 10 MHz s⁻¹. Smith⁹ has noted that none of the models of jovian decametric radiation seems able to account for the S bursts. To explain their negative frequency–time slopes, Ellis⁵ has interpreted them as being due to streams of 2.5 keV electrons travelling outwards along the field lines of Jupiter's magnetic field. Although it was not clear why such streams should always be accelerated outwards, additional evidence for such acceleration is provided by the narrow range of equatorial pitch angles for the radiating electron streams centred on 2.16°, deduced by Ellis to account for the observed variation of df/dt with f . Krausche *et al.*¹⁰ also derived a unique pitch angle in their analysis of S burst radiation. Such pitch angles would be explained if the electron streams were accelerated outwards from Jupiter's ionosphere with an initial pitch angle of 90°. It is easier to think of acceleration mechanisms at Jupiter with initial pitch angles of 90° than of mechanisms at Io leading to equatorial pitch angles of 2.16°.

The modulation of jovian decametric radiation by Io was first demonstrated by Bigg¹. He showed that the probability of receiving emission at Earth is greatly enhanced when Io is near 90° or 240° from SGC. Most of the models proposed to account for the Io effect invoke the Io flux tube, defined by the jovian magnetic field lines passing through the satellite⁹. Current-carrying electrons flow up and down the sides of this tube and emission by these electrons gives rise to the observed radiation. To explain the beaming of the radiation, most models involve mechanisms where the emission is confined to the surface of a cone centred on the field line on which the radiating electron streams are moving. The emission will only be seen when this cone intersects the Earth—this gives rise to the two regions of Io SGC for which decametric emission is observed at Earth.

Previously, the Io effect has been reported only above 20 MHz (ref. 2). The lack of Io effect for bursts with maximum frequency of less than 17 MHz could be explained¹¹ by noting that for all jovian longitudes the gyrofrequencies at the feet of the Io flux tube in both hemispheres are greater than 17 MHz (ref. 12). Electron streams that produce 17 MHz and lower frequency radiation will thus be seen in both hemispheres and will eventually fill the Io L shell by gradient drift and produce radiation independent of the position of Io and of Jupiter.

However, in the case of S bursts, Figs 1 and 2 imply that although bursts with maximum frequency of less than 17 MHz can be observed at all jovian longitudes as expected, they are created only in the Io flux tube. To explain why the electron streams that give rise to S bursts do not populate the Io L shell, we note that Smith⁹ has shown that electrons following field lines in the Io flux tube with energies greater than ≈ 0.8 keV will be swept up by Io. As S burst electrons are deduced to have energies of ≈ 2.5 keV, they will impact upon Io and thus S bursts will only be emitted from the Io flux tube. If the electron streams which give rise to S bursts are accelerated outwards from Jupiter, radiate and are then swept up by Io, this would also explain why only S bursts with negative frequency time slopes are observed.

It is concluded, therefore, that the S bursts are emitted by electron streams that are accelerated outwards from the feet of the Io flux tube in Jupiter's ionosphere. It has been proposed that

these are regions of large magnetic fields (≈ 10 G, ref. 12), large potential differences (≈ 580 kV, ref. 13) and of local ionospheric heating by magnetohydrodynamic waves generated at Io that propagate down the Io flux tube⁹. Such greatly disturbed areas may create instabilities which cause ionospheric plasma to be ejected outwards along the magnetic field lines.

I thank G. A. Gowland and P. Button for technical assistance, and Professor G. R. A. Ellis for valuable discussions.

P. S. WHITHAM

Department of Physics,
University of Tasmania,
Hobart, Tasmania, Australia

Received 7 November 1977; accepted 3 January 1978.

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SM-ND isotopic investigations of Isua supracrustals and implications for mantle evolution

THE ^{147}Sm – ^{143}Nd system has been successfully exploited for the dating of lunar rocks and meteorites^{1–3} and has yielded results of comparable precision to those obtained from the ^{87}Rb – ^{87}Sr system. Furthermore, recent studies of the Angra dos Reis achondrite^{3,4,30} have shown that the age obtained by the Sm–Nd method is in excellent agreement with ages calculated from U–Pb-isotope compositions when the new U-decay constants are used⁵. Ages quoted here are based on the following decay constants: $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$; $\lambda^{147}\text{Sm} = 6.54 \times 10^{-12} \text{ yr}^{-1}$; $\lambda^{235}\text{U} = 0.98485 \times 10^{-9} \text{ yr}^{-1}$; $\lambda^{238}\text{U} = 0.15513 \times 10^{-9} \text{ yr}^{-1}$. Measurements of the Nd-isotope compositions of recent oceanic and continental basalts^{6–11}, combined with estimates of the Sm/Nd ratio of the bulk Earth^{8, 12} have led to a much improved assessment of mantle differentiation^{8, 9, 12}. Hamilton *et al.*¹³ have obtained a Sm–Nd whole rock isochron from Rhodesian Archaean volcanics, which demonstrates the feasibility of dating terrestrial rocks by this method. The latter investigation also indicates that the Sm–Nd system will probably prove more valuable than the Rb–Sr system for dating ancient volcanics, because of the greater stability of Sm/Nd compared with Rb/Sr during alteration processes. Here we report the results of Sm–Nd dating of the oldest known terrestrial rocks from the Isua supracrustal succession in West Greenland.

The geology of the Isua area has been described elsewhere^{14–16}. The supracrustals consist of metasediments and metavolcanics (with associated intrusive sills) and have been deformed into an arcuate belt which is surrounded and locally intruded by Amitsoq gneiss. The sedimentary component includes quartzites, chert, carbonate-rich rocks, semi-pelites, iron formation and an acidic conglomerate unit. The basic volcanics and the cumulate-rich ultramafic igneous rocks have been metamorphosed to low- and medium-grades. Presumed primary mineralogy is only recorded from the olivine-rich ultramafic masses.

Field relations showing intrusion of tonalitic Amitsoq gneiss into the supracrustal succession have been described^{14,15}, demonstrating that at least part of the surrounding gneisses were emplaced after the deposition and first deformation of the supracrustals.

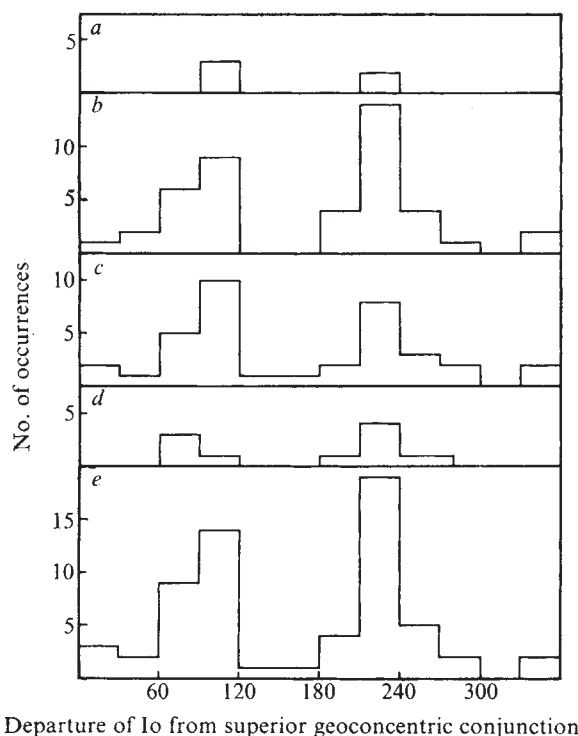


Fig. 2 Number of occurrences of S bursts versus Io's departure from superior geoconcentric conjunction in the frequency ranges: a, 21–24 MHz; b, 14–17 MHz; c, 11–14 MHz; d, 8–11 MHz; e, total.

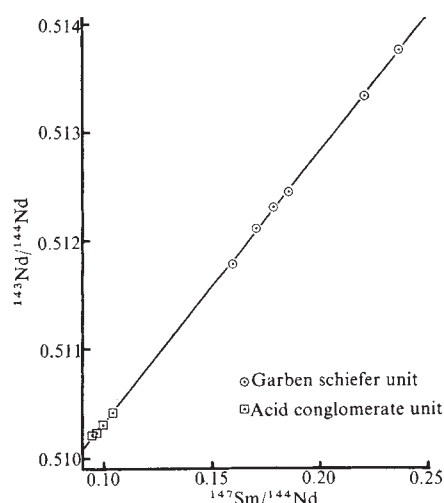


Fig. 1 Sm-Nd evolution diagram for acid (conglomerate unit) and basic (garben schiefer) metavolcanics of the Isua supracrustals ($T = 3,370 \pm 42$ Myr; $(^{143}\text{Nd}/^{144}\text{Nd})_0 = 0.507831 \pm 46$).

The Isua gneisses yield Pb-Pb and Rb-Sr whole-rock ages of about 3,600–3,800 Myr (ref. 17), indistinguishable from those for type Amitsoq gneiss at Godthaab. A discordia age of $3,600 \pm 50$ Myr has been reported^{18–20} for zircons from Amitsoq gneiss. Ages obtained for the Isua supracrustals are mostly within error of the above mentioned ages. A Pb-Pb age of $3,710 \pm 70$ Myr for the iron formation²¹, and Rb-Sr whole-rock ages of $3,710 \pm 90$ Myr for the acid conglomerate unit¹⁷ and $3,740 \pm 60$ Myr on combined data for the acid conglomerate leucocratic schist units²¹ have all been interpreted as the time of deposition and/or metamorphism of the supracrustal sequence^{17,21}. Local well-preserved primary textures suggest that the acid conglomerate unit was largely derived from volcanogenic sediment such as acid tuff, and further that it may be an intraformational horizon with earlier Isua supracrustals (including acid lava flows as the source rock). Zircons from the Isua supracrustals (one sample from a quartzite, the other from the conglomerate matrix) yield $^{207}\text{Pb}/^{206}\text{Pb}$ ages of 3,670 and 3,770 Myr respectively²², whereas U-Pb analyses of single zircons from the conglomerate pebbles give a discordia age of $3,769 \pm 11$ Myr (ref. 20).

The present study is based on basic metavolcanic samples from the garben schiefer unit within the succession, and three pebbles and a matrix sample from the acid conglomerate unit. Analytical procedures used in this laboratory have been reported previously^{9,13}. The Sm-Nd isotope data obtained are presented in Table 1 and shown on a Sm-Nd evolution diagram in Fig. 1. The best-fit line²³ yields an age of $3,770 \pm 42$ Myr (2σ) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (I_{Isua}) of 0.507831 ± 46 (2σ).

Table 1* Sm-Nd data for Isua metavolcanics

Sample	Sm (p.p.m.)	Nd (p.p.m.)	Sm/Nd	$^{147}\text{Sm}/^{144}\text{Nd} \dagger$	$^{143}\text{Nd}/^{144}\text{Nd}$ ($\pm 2\sigma$)
Garben schiefer unit					
171755	0.760	2.556	0.2852	0.1788	0.512309 ± 22
171756	0.2976	0.755	0.3780	0.2371	0.513762 ± 38
171757	1.060	3.985	0.2551	0.1599	0.511783 ± 38
171758	0.365	0.993	0.3525	0.2211	0.513335 ± 34
171759	0.841	2.722	0.2963	0.1858	0.512450 ± 28
171760	0.787	2.774	0.2721	0.1706	0.512116 ± 20
Acid conglomerate unit					
158509	3.757	22.67	0.1589	0.0996	0.510305 ± 38
158510	4.227	26.87	0.1509	0.0945	0.510213 ± 24
158511	4.717	29.43	0.1537	0.0963	0.510230 ± 21
158544	4.516	26.10	0.1659	0.1040	0.510414 ± 26

* $^{146}\text{Nd}/^{144}\text{Nd}$ normalised to 0.7219 (ref. 9). All ratios are atomic.

† $^{147}\text{Sm}/^{144}\text{Nd}$ ratios are measured to a precision of 0.2% (2σ).

Data for the basic rocks only yield an age of $3,772 \pm 134$ Myr (2σ) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50783 ± 17 (2σ). The basic unit is intrusive to the supracrustal succession, yet its Sm-Nd systematics cannot be distinguished from those of the acid conglomerate unit. Thus, although it is doubtful that a simple genetic relationship exists between the two rock suites, they were derived contemporaneously from source regions with indistinguishable $^{143}\text{Nd}/^{144}\text{Nd}$ ratios, and in this sense are consanguineous. Furthermore, the Sm-Nd age obtained is in excellent agreement with the age obtained from single zircons from the acid conglomerate unit²⁰, and provides a precise estimate of the timing of the volcanicity which produced the igneous precursors of the garben schiefer and acid conglomerate units.

The results of the Sm-Nd whole-rock isochron studies of the Isua and Rhodesian greenstone belt volcanics¹³ are compared with the Sm-Nd data of DePaolo and Wasserburg^{7,8} in Fig. 2. Although the latter data are based on single whole-rock analyses with the age derived from a different parent-daughter system and in some cases from different samples, there is a large measure of consensus for an early terrestrial development of Nd in an environment with an approximately chondritic Sm/Nd ratio^{7,8}. This contention derives from the fact that all the data points lie close to the growth line in Fig. 2, representing the evolution of a reservoir which had a $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50682, 4550 Myr ago³ and a Sm/Nd ratio of 0.308, the average chondrite value³⁰. The actual Sm/Nd ratio required to generate I_{Isua} at 3,770 Myr ago is 0.306 ± 0.006 (as measured now).

It has been suggested that a number of refractory elements such as Sm and Nd and other rare earth elements, U and Sr, are present in the Earth in chondritic proportions^{12, 24–27}. It has also been noted that systematic volatile element fractionations exist between the Earth, the Moon and chondrites and that volatile to refractory element ratios (such as, Rb/Sr, K/U and Pb/U) are substantially lower in the Earth and Moon than in carbonaceous chondrites. The observed covariation of Nd- and Sr-isotope composition for present-day basalts has been used to suggest a Rb/Sr ratio of ~ 0.03 for the bulk Earth^{8,9}. Sm-Nd data for Archaean rocks (Fig. 2) lend support to this estimate. Recently, however, Hart and Brooks²⁸ have suggested that the Earth initially had a chondritic Rb/Sr ratio that was subsequently reduced by a removal of Rb either into the core or by degassing.

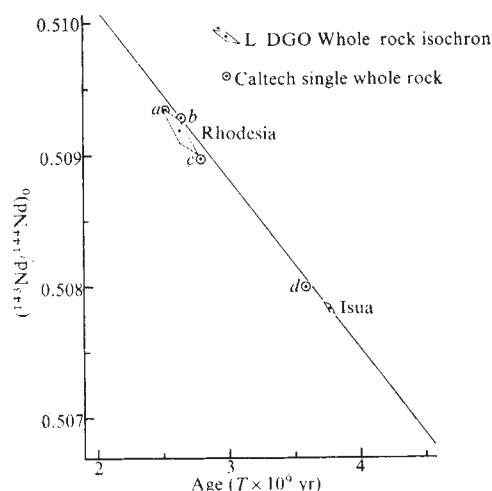


Fig. 2 Initial $^{143}\text{Nd}/^{144}\text{Nd}$ against age T for Archaean igneous and meta-igneous rocks, compared with a chondritic reservoir. The line drawn is for a reservoir which had a $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50682, 4550 Myr ago (equal to that in Angra dos Reis³) and evolved with a Sm/Nd ratio of 0.308 (equal to the chondrite average). The Caltech data plotted are *a*, Great Dyke; *b*, Preissac-Lacorne granodiorite; *c*, Fiskensæset anorthosite; *d*, Amitsoq gneiss. The initial value for Isua (I_{Isua}) requires that Sm/Nd = 0.306 ± 0.006 (as measured today) during the interval 4,550–3,770 Myr.

The current paucity of precise initial $^{87}\text{Sr}/^{86}\text{Sr}$ isotope data complementary to the initial $^{143}\text{Nd}/^{144}\text{Nd}$ data makes the hypothesis of Hart and Brooks²⁸ difficult to substantiate.

The Sm–Nd age of $3,770 \pm 42$ Myr and initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.507831 ± 46 obtained from the garben schiefer and acid conglomerate units at Isua further demonstrate the potential of the Sm–Nd system for whole-rock dating of ancient metavolcanics, and for evaluating the nature of mantle differentiation.

The analytical work has been supported by US NSF grants EAR-75-20840 and EAR-76-03802. We thank Genevieve Wesselman for technical help. The samples were collected during field work sponsored by the Geological Survey of Greenland.

P. J. HAMILTON
R. K. O'NIONS
N. M. EVENSEN

Lamont-Doherty Geological Observatory
of Columbia University,
Palisades, New York 10964

D. BRIDGWATER
J. H. ALLAART

Geological Survey of Greenland,
Ostervoldgade 10,
Copenhagen K,
Denmark

Received 14 November 1977; accepted 5 January 1978.

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Sand deserts during glacial maximum and climatic optimum

THE past 20,000 yr have witnessed tremendous climatic changes, a glacial maximum at about 18,000 yr BP and a climatic optimum centred on about 6,000 yr BP, both of which mark extreme situations for the Quaternary. This paper attempts to show that active sand dunes were extensive 18,000 yr ago. Conversely, it seems that sand dunes were generally dormant 6,000

yr ago. Thus the former textbook concept^{1,2} of an arid climatic optimum and a pluvially active glacial maximum is reversed.

Climatic conditions are largely characterised by temperature and wetness³. Temperature distribution on the Ice-Age Earth has been extensively studied by the CLIMAP group^{4–6}, and their data base was used in numerical modelling by Manabe and Hahn⁷, who also included the distribution of sea-surface temperatures, glaciers, land albedo, sea-land and so on in their data base and thereby simulated the distribution of wetness (precipitation minus evaporation) and surface wind systems. The fossil extent of sand deserts provides an excellent test for the numerical climate models, the predictions of which are generally confirmed.

The maps of dune activity for the two climatic extremes rely almost exclusively on published evidence dated by radiocarbon and U-series methods. Archaeological evidence was seldom admitted. The ^{14}C dates (uncorrected) are mainly derived from shells, bones, charcoal, wood chunks, palaeosoils and from lake deposits with pollen profiles. Only key articles are cited here, but a discussion of some 350 references is given in refs 8 and 9. All the usual reservations about ^{14}C -dating of such objects apply, but this does not interfere with my overall conclusions. The dunes or dune fields recognised in the present compilation do not usually contain datable material, but they were bracketed by dated marker beds below and above.

Modern active inland sand dunes occur in the arid deserts of the Horse Latitudes and in some arid mid-latitude and high-latitude regions of continental interiors, in rain shadow deserts¹⁰ and polar deserts¹¹ (Fig. 1a). Today about 10% of the land area between 30°N and 30°S is covered by active sand deserts.

Regardless of their morpho-type, all active inland sand dunes indicate aridity, as is readily apparent from comparison with a present-day wetness map³ and the distribution of a 25-mm precipitation isoline (Fig. 1a). Sand dunes also indicate strong winds¹², and wind directions can be read from their external and internal structures (see refs 13–15).

Sand dunes and associated deserts were much more widespread 18,000 yr ago than they are today (Fig. 1b). They characterised almost 50% of the land area between 30°N and 30°S, forming two vast belts. In between, tropical rain forests and adjacent savannahs were reduced to a narrow corridor, in places only a few degrees of latitude wide^{17–19}. This fits the concept of 'ice-age aridity' in the tropics^{20–25} which has recently replaced that of 'ice-age pluvials'^{11,2,26}. This paper also extends ice-age aridity into the middle and higher latitudes, with important implications for plant and animal ecology.

The time slice in Fig. 1b is about 2,000 yr thick, and includes the somewhat delayed aridity climax of the Arabian and Australian deserts (about 17,000 yr BP)^{12,27,28}.

The striking widening of the sand-dune belts towards the equator and the derived palaeo-wind directions generally confirm the increased tropical aridity (Fig. 2) and the modelled wind patterns⁷ predicted by the numerical climate simulation of Manabe and Hahn; for example, sand dunes expanded over the tropical margin of the Sahara^{21,23,29}, southern Arabia²⁷, north-west India^{13,30,31} and the northern part of South America^{17,32}. No dates of dunes were found to support the model's simulation of drier conditions in Bengal and Burma. In the Southern Hemisphere, the most obvious examples lie in the Kalahari desert, South Africa and the Congo Basin^{8,24,33–35}, and in the deserts of North Australia²⁸ and East Brazil¹⁷.

As compared to the subtropics, information on dune-field development is hampered or confused in deserts of Mediterranean latitudes, because the periods of extreme aridity were much shorter. Hence the concept of synglacial pluvials remained valid for many of these regions (see refs 22, 23, 36, 37). A careful re-examination of all published ^{14}C -dated sources⁹ showed that in the Southern Hemisphere the dune fields and marginal dry steppe regions advanced polewards of 35°S in South Australia^{12,28,38}. The Kalahari sands possibly were in part driven southwards by an anticyclonic wind pattern toward the Orange and Vaal Rivers (28°S), and only the margins of

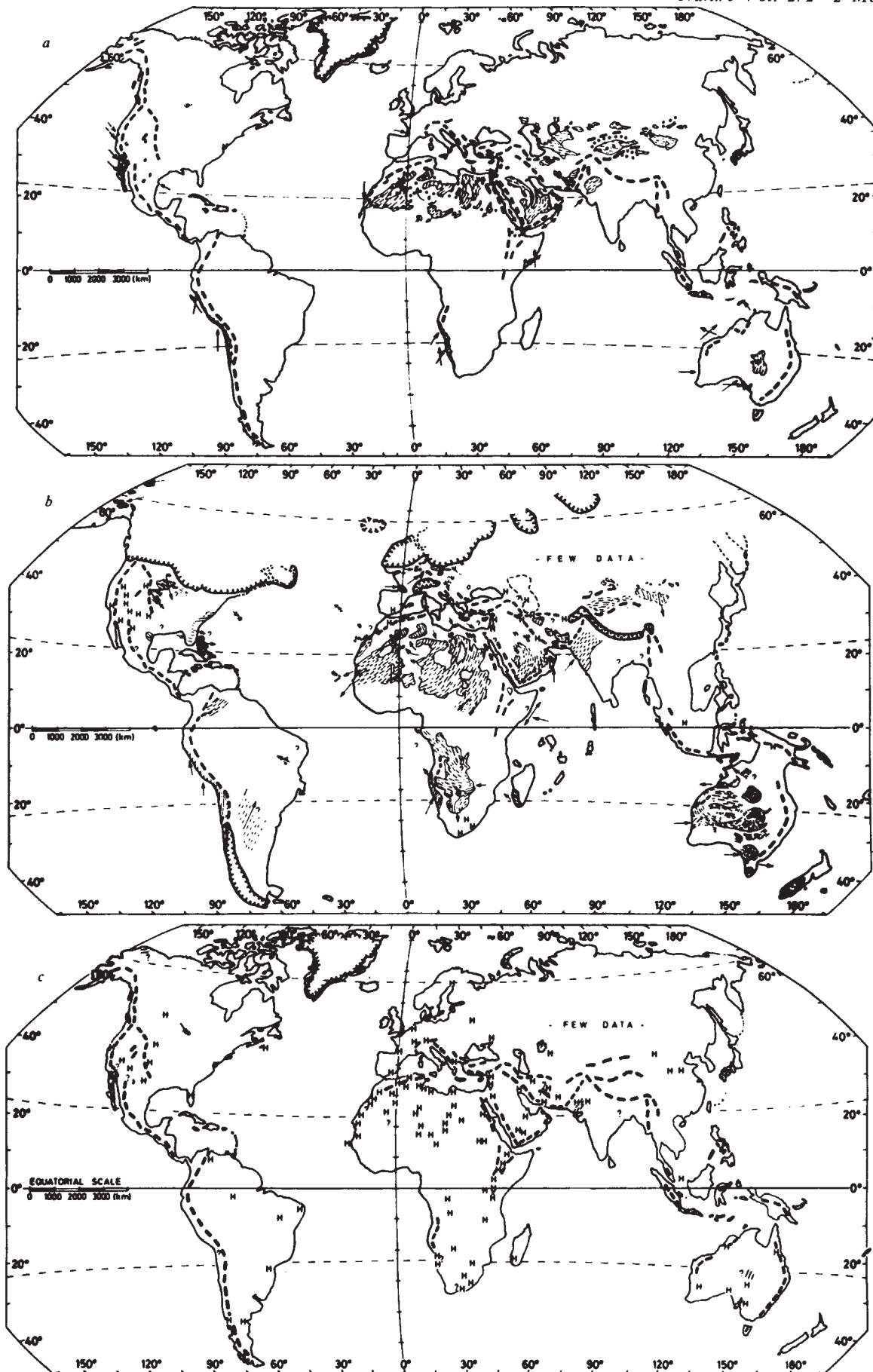
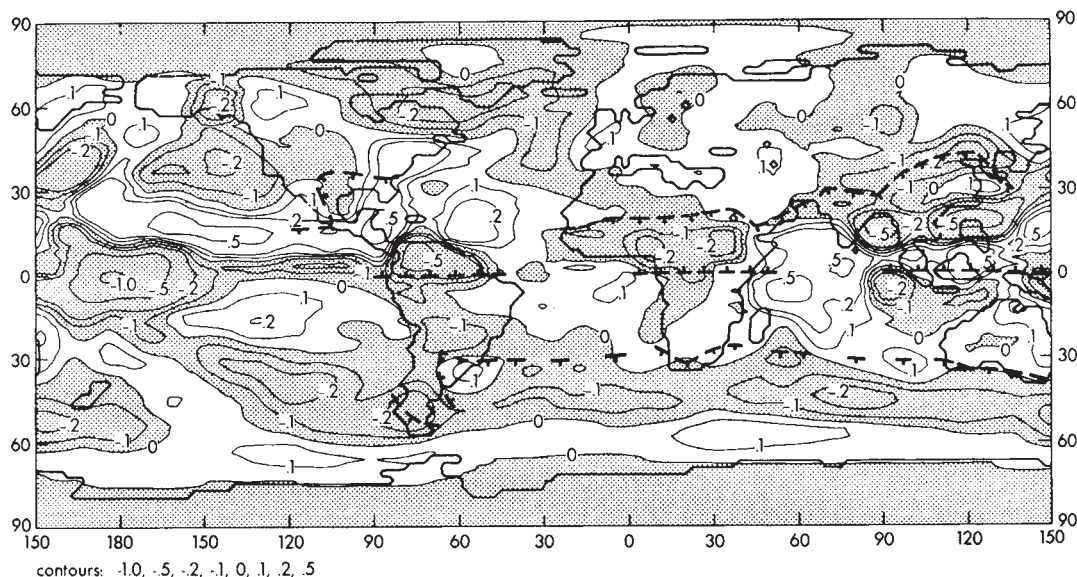


Fig. 1 Active dune fields and long-term resultant effective wind regimes for the present (a), 18,000 (b) and 6,000 (c) yr ago. Continental outlines at 18,000 BP (Fig. 1b) represent sea-level lowering of 120 m (ref. 16). Hatched areas indicate dune fields and major dune contours. Arrows are long-term resultant surface-wind directions. Dotted lines in Fig. 1a mark areas with less than 25 mm annual precipitation. H signs stand for fossil wet conditions excluding dune formation. Heavy dashed lines indicate major mountain bars, heavy serrate lines surround ice caps². Fig. 1a is partly based on ref. 10.

Fig. 2 Geographical distribution of simulated net water supply (rainfall + snowmelt - evaporation = wetness) (cm d^{-1}) as difference of ice-age experiment minus standard (present) experiment; for June-August season only⁷. Horizontal grid resolution ~ 270 km. Heavy solid lines are continental outlines. Heavy dashed lines encircle zones with bulk precipitation during June-August. The assistance of Drs Manabe and Hahn in interpretation is acknowledged.



South Africa remained humid (Fig. 1b)^{14,34,35,30}; northern Patagonia, Paraguay and south-west Brazil were also reportedly dry and dune covered⁴⁰⁻⁴².

An analogous trend probably holds true for North Africa, where sand dunes spread out at 18,000 yr BP from south of Lake Chad (10°N) to the Atlas Mountains and to near Algiers at 37°N. (This view differs from that of Faure⁴³ and Street and Grove²², who place the general maximum of aridity between 15,500 and 13,000 yr BP). For example, maximum dune activity lasted in the Nile Valley, close to Wadi Halfa and in the nearby Dungul oasis (23°30'N), from more than 19,000 to 17,800 and/or 16,500 yr BP⁴⁴⁻⁴⁶. South of the Atlas, the major phase of the sand desert 'Grand Erg Occidental' was pinpointed in the Saoura Valley (30°N) as between 19,800 and 17,510 yr BP, and the end of Erg Chech (26°N) activity as 17,700 yr BP³⁷. Seif dunes off and in North Cyrenaica (32°N) were active before 16,300 yr BP^{47,48}. Mobile dunes close to Algiers^{49,50}, gypsum crusts in the southern piedmont region of the Atlas Mountains^{50,51} and Mediterranean loess, pollen and dune profiles⁵²⁻⁵⁴ apparently belong to the same 18,000 yr BP time slice.

Thus the dune-forming aridity event lasted some 2,000-3,000 yr in the northern Sahara and some 6,000-8,000 yr in the southern Sahara. This event was closely preceded and followed by cool lake-forming periods, which probably in the north erroneously gave rise to the theory of the synglacial pluvial^{22,25,26}.

A general onset of wetness in the South Sahara is found only after 12,500 yr BP, after 15,500 yr BP in mountainous snowy regions like the Tibesti⁵⁵ and after 17,700 yr BP in the Algerian North Sahara. The Kalahari dune sands (20°S) were stabilised at 17,300 yr BP^{55,39}. Accordingly, the end of extreme aridity and the first signals of wetter conditions seem to be fairly synchronous in the poleward margins of the North and South African deserts.

The moderately wet conditions suggested at 18,000 yr BP for mountainous regions of the Eastern Mediterranean⁵⁶, the Caspian Sea and the Caucasus and Hindukush Mountains⁵⁷⁻⁵⁹ can be related to snow coverage. At this time, the Persian Gulf region⁸ was dry and the Zagros Mountains were drier than they are today⁶⁰. Loess and sand-desert stratigraphy⁶¹⁻⁶³ indicates cold sand deserts and strong loess storms for the Inner Asian deserts extending from Iran to Mongolia (in contrast to the views of Krinsley⁶⁴).

The mid-latitude climate of pleniglacial North America is also controversial. Well-dated cool pluvial lakes west of the Rocky Mountains^{65,66} contrast with synchronous sand deserts and Peoria loess deposition east of these mountains from Nebraska and Kansas to Illinois⁶⁷⁻⁷⁰. A boreal forest separated

these northern-midwest deserts from other dune fields in south-west Texas and from Florida to North Carolina⁷¹⁻⁷³.

The dunes and associated loess deposits in the northern Midwest were formed by northwesterly winds surrounding the Laurentide ice sheet^{14,68,69}, and are comparable to the permafrost-influenced Alaskan polar deserts¹¹. A similar situation obtains for the widespread European sand dunes near the Scandinavian ice cap^{74-77,15}.

The pattern of the Holocene climatic optimum (¹⁴C-dated 6,000 yr BP) (see refs 6, 18, 78-82) was radically different from that of 18,000 yr ago (Fig. 1c): The only active, but minor dune fields were reported from North Alaska¹¹, the Great Basin of North America^{65,66}, Canada and North Minnesota⁸³ and the Indian Thar desert³⁰; questionable fields from the Iranian and Central South Australian deserts⁹. On the other hand, at some 100 key localities in former deserts all over the world (H symbols on Fig. 2c), ¹⁴C-dated outcrops establish humid environments such as that of soil formation, which exclude dune activity⁹.

The 6,000 yr BP humidity (see refs 18, 56, 84 and lively Saharan drawings in ref. 85) lasted only from about 6,500 to 5,500-5,000 yr BP, and was preceded by at least two earlier periods of widespread wetness from 12,500 to 11,000 and from $\sim 10,000$ to 7,500 yr BP^{12,22,29,86}. Although it does not show the maximum of humidity everywhere, the 6,000 yr BP time slice was chosen because it parallels a general temperature maximum, and monsoonal summer rains probably reached their most northerly position at $>30^\circ\text{N}$ in Lybia and the Atlas Mountains^{50,87}, and the Hoang Ho Ho province⁶³. In contrast, the early Holocene pluvials were generated by west winds during winter⁸⁷ and paralleled considerable temporary dune activity in the northern Sahara²³.

The numerical experiments⁷ suggest that the tropical sea-surface temperature anomalies affect not only the oceanic climate, but also precipitation, evaporation and dominant wind directions on land. The fluctuations of sand deserts support these observations for the lower latitudes of Africa and South America. Here, a considerable contraction of the inter-tropical convergence zone (ITCZ) was suggested for 18,000 yr BP⁸⁸, and desert expansion amounts to some 500 to 900 km symmetrically and synchronously in the Northern and Southern Hemisphere (Figs 1 and 2). The numerical model seems less valid in the mountains and land-locked interiors of the northern continents, where the albedo of extensive ice and seasonal snow fields can strongly influence atmospheric circulation (such as in the Asian monsoon region)⁷.

A global general reduction of wetness 18,000 yr ago is illustrated by greatly expanded dune fields—also outside the

subtropics. Presumably, lowered temperatures also resulted in strongly diminished evaporation, but this could not balance the drop in precipitation and the strengthened winds. Conversely, the increased evaporation due to the temperature maximum at 6,000 yr BP could not overcompensate the intensified precipitation. Thus, changes in precipitation seem to be a driving function controlling the distribution of sand dunes.

The expanse of sand deserts 18,000 yr ago must have considerably increased the Earth's albedo, already high because of the extended ice caps. A first major reduction of deserts and albedo clearly preceded the onset of full deglaciation (around 15,500 yr BP⁸⁹), by about 1,000–2,000 yr.

Published data suggest that climatic changes from desert to vegetated land and vice versa are very rapid, spanning no more than a few hundred years. One example is the modern West Sahara, whose dune fields became active no more than 300–400 yr ago⁹⁰ and where Portuguese explorers still observed summer rains at 24° (today hardly reaching 19°N)⁹¹. Many of the short desert phases could easily remain unrevealed in the terrestrial geological record⁸.

Present dune patterns resemble those of the last pleniglacial rather than those of the last climatic optimum. The widespread cool pluvials of the foregoing interstadial before 10,000–25,000 yr BP^{8,23} (during marine Oxygen Isotope Stage 3) perhaps display a third fundamental alternative. If a close inter-relationship between low sea-surface temperatures and continental aridity holds true for small climatic fluctuations as well as large ones, the present drop in sea-surface temperature in the Northern Hemisphere⁹² might be interpreted as an early signal for drought within continents.

MICHAEL SARNTHEIN

Geologisch-Paläontologisches,
Institut der Universität,
D-2300 Kiel, FRG

Received 21 November; accepted 10 December 1977.

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Factors controlling oxygen isotopic composition of fallen snow in Antarctica

TRANSPORTATION of water vapour to the Antarctic ice sheet is one of the most important factors controlling oxygen isotopic composition of Antarctic snow, firn and ice. In previous studies^{1–5}, the isotopic composition of precipitation was related only to its temperature of formation. In the study reported here, the relationship between the oxygen isotopic composition of fallen snow at the Syowa Station and the transportation process of water vapour to the Antarctic ice sheet has been investigated. It was found that the oxygen isotopic composition of fallen snow is largely controlled by the supply of ¹⁸O-rich water vapour resulting from the approach of a cyclone, and is strongly related to the distance from the open sea to the sampling station.

Snow samples were collected at the Syowa Station in 1974 by the research group of the 15th Japanese Antarctic Research Expedition 1973–1975 led by O. Watanabe (ref. 6). The station is located on East Ongul Island off Prince Olav Coast, at lat 69° 00' S and long 39° 35' E. The snow samples were kept in polyethylene bottles, transported to Japan under refrigeration and melted only just before the oxygen isotope determination.

The oxygen isotopic composition was measured for 38 samples of fallen snow, 16 samples of which include partly drifting snow, and four samples of drifting snow. The experimental procedures are essentially the same as those described by Epstein and Mayeda⁷. The ¹⁸O/¹⁶O ratio of CO₂ equilibrated with melted snow was measured with a double collector mass spectrometer (Varian Mat CH-7). Analytical results are expressed in δ¹⁸O_{SMOW} notation⁸ and analytical error is ±0.2‰.

Figure 1 shows the variation of oxygen isotopic composition of snow and meteorological conditions⁹ during 1974. A

seasonal variation of $\delta^{18}\text{O}$ is obvious. The variations of $\delta^{18}\text{O}$ in drifting, and fallen snow partly including drifting snow, seem to follow the same seasonal trend as that of fallen snow without drifting snow. The seasonal variation of $\delta^{18}\text{O}$ seems to follow that of monthly mean surface air temperature, except for August.

The $\delta^{18}\text{O}$ values of fallen snow collected at the King Boudouin Station^{1,2} near the Syowa Station in 1958 and at the South Pole⁵ are grouped into 'isotopic summer' (November–April) and 'isotopic winter' (May–October) by abrupt jumps in the variation of $\delta^{18}\text{O}$. Picciotto *et al.*² pointed out that two periods of such abrupt jumps of $\delta^{18}\text{O}$ correspond very well to those of the abrupt jumps in the variation of monthly mean air temperature of the middle troposphere, where the precipitating clouds are formed.

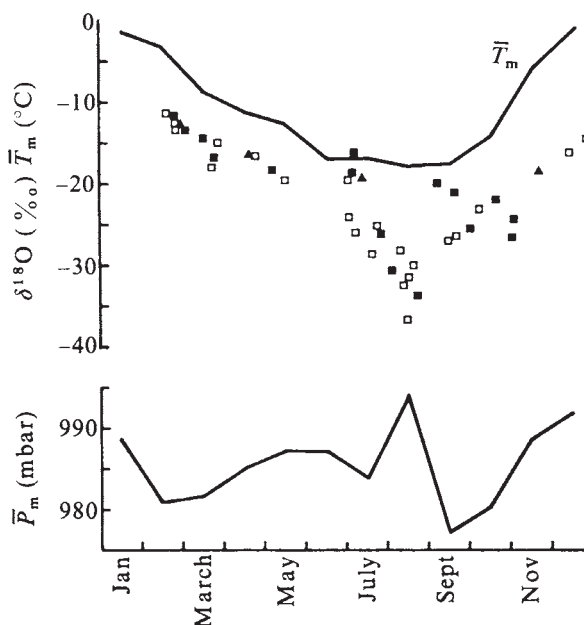


Fig. 1 Variations of oxygen isotopic composition of snow ($\delta^{18}\text{O}$), monthly mean surface air temperature ($\bar{T}_m$) and monthly mean atmospheric pressure in station level ($\bar{P}_m$) during the course of 1974, at the Syowa Station. □, Fallen snow without drifting snow; ■, fallen snow partly including drifting snow; ▲, drifting snow.

Two abrupt jumps are observed between March and April and between October and November in the variation of monthly mean air temperature of the middle troposphere above the Syowa Station in 1974 (ref. 9). However, the 'isotopic summer' of 1974 at the Syowa Station did not begin from May as observed at the King Boudouin Station in 1960³. This suggests that the temperature of formation of precipitation is not the main factor controlling its isotopic composition.

Figure 2 shows the isotopic composition of snow with respect to the temperature range in the corresponding cloud layer (determined as the layer with relative humidity of 100% from the surface synoptic, and the aerological data). Drifting snow samples were excluded. The remarkable relationship between $\delta^{18}\text{O}$ of precipitation and its temperature of formation as observed at the King Boudouin Station² is not seen in this figure. This supports the idea that the oxygen isotopic composition of precipitation is not controlled mainly by its temperature of formation.

Figure 3 shows the variations of oxygen isotopic composition of fallen snow and meteorological conditions⁹ in July and August 1974. The variation of $\delta^{18}\text{O}$ during 1–7 July and 12–21 August follows faithfully that of daily mean surface air temperature. But this does not mean that $\delta^{18}\text{O}$ of precipitation is controlled mainly by its temperature of formation.

In these two periods, a cyclone passed, and surface air temperature and wind velocity increased and decreased with decreasing and increasing atmospheric pressure respectively, as seen from Fig. 3. Therefore, the $\delta^{18}\text{O}$ of fallen snow increases under cyclonic conditions under strong wind. On 2 August, both surface air temperature and wind velocity increased under an anticyclone, whereas $\delta^{18}\text{O}$ did not increase. This indicates that $\delta^{18}\text{O}$ of precipitation formed under cyclone is higher than that formed under anticyclone, even at the same temperature of formation of precipitation. This is shown in Fig. 2. $\delta^{18}\text{O}$ of precipitation is considered to be increased by the supply of ^{18}O -rich water vapour due to the approach of a cyclone.

The temperature of formation of fallen snow is not as extraordinarily low in August as $\delta^{18}\text{O}$ of fallen snow suggests (see Fig. 2). The amount of ^{18}O -rich water vapour supplied by cyclone is smaller in August, which has a very high atmospheric pressure as seen in Fig. 1, than in the other months. Hence it becomes understandable why $\delta^{18}\text{O}$ of fallen snow is so low in August. The variation of $\delta^{18}\text{O}$ of precipitation is caused not only by its temperature of formation but also by the supply of ^{18}O -rich water vapour resulting from the approach of cyclone.

Taking into consideration the supply of ^{18}O -rich water vapour from the cyclone, Fig. 2 shows that $\delta^{18}\text{O}$ of fallen snow in December–May is higher than that in July–November, even at the same temperature of formation of fallen snow. This means that supplied water vapour is richer in ^{18}O in December–May than in July–November. This is considered to be strongly related to the shorter distance from the coast of open sea to the sampling station in December–May than in July–November. The distances determined from the pictures of ARTS and ESSA are ~40–400 km between December and May and ~800–1,100 km between July and November. The variation of the distance shows two abrupt jumps between May and July and between November and December, as Treshnikov¹⁰ pointed out. The difference of transportation process of water vapour between the two above periods may cause the appearance of 'isotopic summer' and isotopic winter'.

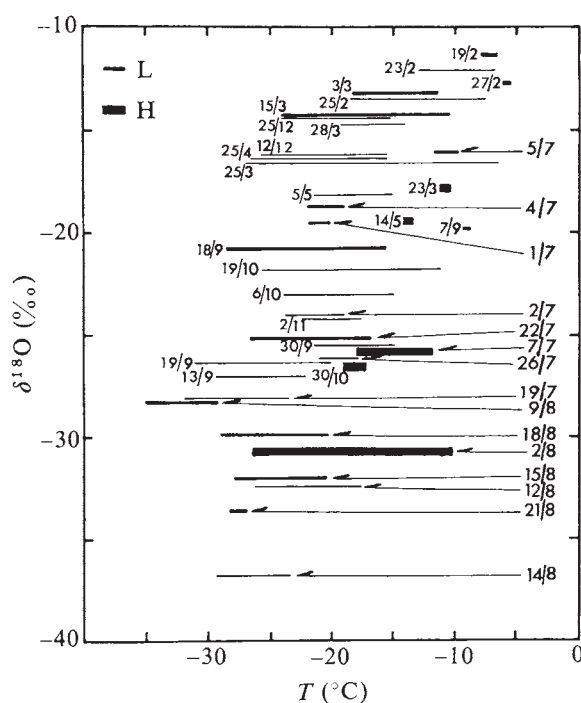


Fig. 2 Oxygen isotopic composition of fallen snow at the Syowa Station with respect to the temperature range in the corresponding cloud layer. Date of collection in July and August and in the other months are given on right side and on left side, respectively. H, Under anticyclone; L, under cyclone.

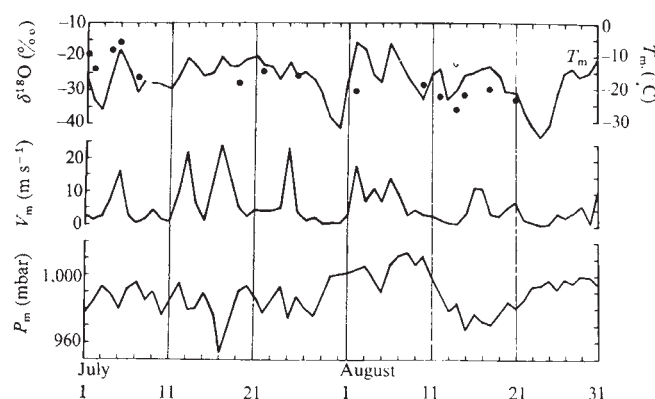


Fig. 3 Variations of oxygen isotopic composition of fallen snow (●), daily mean surface air temperature (T_m), daily mean wind velocity (V_m) and daily mean atmospheric pressure in station level (P_m) in July and August 1974, at the Syowa Station.

The variation of isotopic composition of precipitation is controlled not only by its temperature of formation, but also by the transportation process of water vapour to the Antarctic ice sheet. Due to the sparsity of the data, further samples are being collected.

I thank O. Watanabe (Water Research Institute, Nagoya University) for the collection of samples, S. Oana, N. Nakai and Y. Mizutani (Department of Earth Sciences, Nagoya University) for their help in the oxygen isotope determination, and Y. Kitano and K. Higuchi (Water Research Institute, Nagoya University) for their comments.

KIKUO KATO

Water Research Institute,
Nagoya University,
Chikusa, Nagoya 464, Japan

Received 30 August 1977; accepted 5 January 1978.

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Geostimulation of root growth and pigment synthesis in mustard seedlings

GRAVITY as an environmental factor affecting plant growth is generally considered to determine the direction of growth. However, an effect of gravity on the rate or intensity of growth—referred to in the older literature¹ as a geotonic, as distinct from a geotropic, effect—is also observable, as in, for example, the decreased growth rate of an inverted shoot. Geotonic effects have not received the attention given to tropic responses, possibly because of the experimental difficulty of clearly

separating one from the other. Ideally, the tonic effect of gravity on growth activity should be demonstrable on some expression of growth unconnected with a geotropic correction. In the experiments reported here on the effect of gravity on the growth of mustard seedlings, we have observed a tonic effect of gravity on root growth and pigment synthesis.

Seeds of white mustard, *Sinapis alba* L., were moistened with 0.1 mM CaCl_2 , pH 6.5, and left to swell for about 4 h. Seeds of uniform size were then selected and placed on circles of Whatman No. 1 filter paper in 140-mm plastic Petri dishes containing CaCl_2 solution. The treatments consisted of one horizontal dish, two dishes clamped at angles of 45° and 85° to the horizontal, and one dish which was rotated horizontally at 300 r.p.m. on a spinner constructed in the Institute workshop. On the upright dishes, the seeds were positioned to form a row parallel to the horizontal plane. Seeds were positioned in a circle with a radius of 15 mm on the spinning dish, where they were subjected to a force of 1.5g. All treatments were set up in the light and then kept in the dark at 22 °C for a further 44 or 68 h. An additional series of the 'tonus' treatments was set up with seeds which had imbibed on the horizontal plane for 24 h. From Table 1, it can be concluded that root growth is promoted by an applied longitudinal force which is perceived by the germinating seed before radicle emergence. Although not shown in Table 1, seedlings which are transferred from a vertical to a horizontal plane have a decreased rate of root growth relative to seedlings left to grow in a vertical plane.

A similar pattern of results is obtained from seedlings continually exposed to white light (Table 2), although here the roots on the horizontal plane are more inhibited relative to those growing on the vertical plane, because of the greater sensitivity of horizontal roots to light inhibition².

However, the most striking difference between light-grown horizontal and vertical seedlings is the more rapid development of pigment, both anthocyanin and chlorophyll, in the vertical seedlings. The pattern of pigment synthesis in vertical and horizontal seedlings over the period 30–70 h from wetting of the seed is shown in Fig. 1. Some values from horizontal seedlings subjected to a force field of 1.5g are also shown, confirming that a longitudinal force approximating to the terrestrial value can, when applied on a horizontal plane, reproduce the effect of gravity.

These results illustrate a tonic effect of axial force on root growth and on pigment synthesis. Smaller effects of gravity on root growth have already been reported^{3–5}. Burstrom⁵ observed that the growth rate of horizontal wheat roots was 10% less than that of vertical roots, and attributed the difference to a tonic effect of gravity.

Larsen⁴ had earlier noted a similar effect with *Artemisia* roots. He took the view that the differential growth rate was caused, not by gravity promoting vertical growth, but rather by the inhibition of horizontal growth by gravity when acting perpendicularly to the root axis. More recent experiments showed root growth under conditions of freefall⁶ to be retarded relative to vertical roots. The experiments reported here, although not excluding the possibility of an inhibitory effect of gravity on horizontal growth, emphasise the positive effect on root growth of an applied longitudinal force of magnitude

Table 1 The effect of time of application of geostimulus on the root growth of mustard seedlings in the dark

Angle subtended with the horizontal	Time of geostimulus application	0°	45°		85°		Horizontal spinning	
		—	4 h	24 h	4 h	24 h	4 h	24 h
Growth after	48 h	13.6±1.28	27.4±1.48	20.3±1.35	36.2±1.25	24.0±1.05	40.0±1.65	24.9±0.82
Growth after	72 h	38.4±2.06	64.9±2.36	48.7±1.74	73.8±2.37	59.9±2.09	81.6±3.06	67.5±1.94

Thirty seeds were placed in Petri dishes as described in the text, after having been wetted and left to imbibe on the horizontal plane for 4 h or 24 h. The upright dishes were clamped at the angles shown. The rotated dish was spun at 300 r.p.m. Dishes were kept in the dark at 22 °C. Root length was measured 48 h after wetting. The values shown are the mean length in mm±s.e.m. A further set of measurements were made at 72 h on a duplicate set of dishes.

Table 2 Comparison of the growth rate of roots of mustard seedlings grown horizontally and vertically in the light and in the dark

Time of measurement	Horizontal		Vertical	
	Dark	Light	Dark	Light
48 h	11.0±0.87	6.1±0.60	22.0±1.13	14.2±1.26
72 h	38.8±3.14	17.8±1.94	61.5±3.45	39.7±2.73

Seeds were wetted and allowed to imbibe on the horizontal plane for 24 h. Thereafter, seeds at a similar stage of development were selected and placed in Petri dishes as described in the text. Illuminated dishes were exposed to white light from fluorescent tubes, yielding a photon flux density of $150 \mu\text{erg m}^{-2} \text{s}^{-1}$ at the level of the seeds. The values shown are the mean length in mm of 30 roots $\pm$ s.e.m. The 48-h and 72-h measurements were made on duplicate sets of seedlings.

up to the terrestrial value of 1g. Experiments with higher centrifugal speeds indicate very little additional enhancement of root growth.

The earlier formation of pigment in geostimulated seedlings is particularly interesting. Anthocyanin production in mustard is under phytochrome control and, further, is dependent on the synthesis of phenylalanine ammonia-lyase and possibly other enzymes⁷. Thus the question arises as to whether gravity influences developmental processes by an effect on phytochrome, leading to the regulation of enzyme activity, or more directly by affecting the functioning of the enzymes themselves. The possibility of a direct link between enzyme activity and the gravity-sensing system in the wheat node has already been suggested⁸, and the results reported here offer further justification for that view.

The positive effect on pigment development of the longitudinal component of an applied force points to a preferred orientation

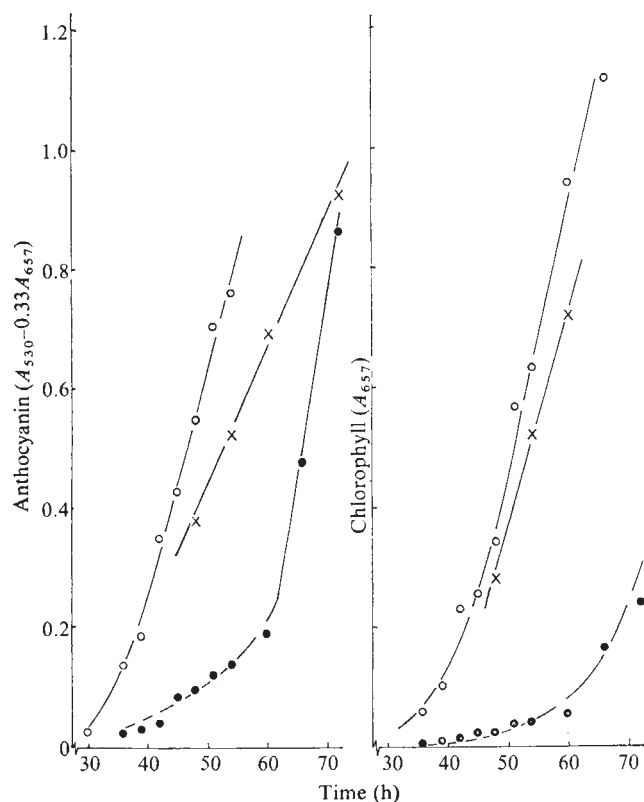
of cellular components for maximum growth activity. Although a polar movement, under the influence of gravity, of regulatory compounds to or from the cotyledons and hypocotyl is possible, the acceleration of pigment formation in geostimulated seedlings does not seem to depend on transport from the root, for excision of the radicle at the earliest practical time does not prevent the accelerated development. On the other hand, regulation by an axial force is not irreconcilable with the current view that the mode of action of phytochrome in morphogenesis has to do with specific alterations in membrane permeability, resulting in the release of hormones, enzymes and substrates⁹. The influence of gravity on plant morphogenesis is currently attracting renewed interest¹⁰. Regulation of phytochrome and of enzyme activity by gravity may well be involved in the overall control of plant growth and merits further investigation.

I. R. MACDONALD
D. C. GORDON

*The Macaulay Institute for Soil Research,
Craigiebuckler, Aberdeen, UK*

Received 30 August 1977; accepted 13 January 1978.

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Mercury in fish and waters of the Irish Sea and other United Kingdom fishing grounds

THE Ministry of Agriculture, Fisheries and Food has reported high mercury (Hg) levels in marketable fish from several United Kingdom fishing grounds^{1,2}. My studies (Table 1) indicate accompanying high dissolved Hg concentrations in waters within the same study areas. An analysis of both sets of data has revealed a linear relationship (correlation coefficient 0.92) between the mean Hg concentrations of the water and fish. In distant waters a concentration factor of Hg from water to fish was 2.9×10^3 compared with 10.6×10^3 in coastal waters between Anglesey and St Bees Head. Fish harvested from these industrially affected waters ($> 50 \text{ ng l}^{-1}$ mean dissolved Hg) contained more than the 0.5 p.p.m. Hg limit set for edible fish in many countries.

The distribution of dissolved Hg during spring and summer months in the Irish Sea has been reported³. Figure 1 shows the less patchy distribution found in a subsequent investigation of Hg levels in surface waters during January 1974. Sixty-six stations were occupied and the samples were collected, treated and analysed as before⁴.

Low levels ($< 10 \text{ ng l}^{-1}$) were observed in shallow waters

Table 1 Boundaries of sample areas and the mean levels of Hg in water and fish sampled

Boundaries of sample areas	No. of water samples	Hg conc. in water ng l^{-1} *		No. of fish samples	Hg conc. in fish mg kg^{-1}		Water to fish concentration factor $\times 10^3$
		Mean	Range		Mean†	Range†	
A Portland Bill to St Austell Bay‡	2	18.0	15–21	214	0.16	0.01–0.66	8.9
A' St Austell Bay to Hartland Point‡	4	20.5	10–35	69	0.18	0.03–0.90	10.0
B Hartland Point to St David's Head‡	22	30.5	9–70	246	0.19	0.01–1.20	9.3
B' St David's Head to Anglesey	20	34.0	0–170	119	0.17	0.02–0.68	8.3
C Anglesey to Formby Point	63	47.3	4–209	163	0.23	0.03–1.20	7.5
C' Formby Point to St Bee's Head	48	51.4	4–443	179	0.17	0.02–0.76	5.6
D Solway	1	40.0	—	64	0.26	0.05–0.66	7.7
E Central Irish Sea	18	16.6	0–45	28	0.55	0.26–1.50	11.6
I Off Belfast	4	27.8	7–40	239	0.51	0.05–2.40	9.9
J South of Belfast	11	20.5	15–173	36	0.24	0.06–0.58	6.0
K Southeast of Ireland	17	36.9	15–173	71	0.34	0.05–0.91	20.5
L Mersey Estuary	44	54.9	5–132	54	0.10	< 0.01–0.31	3.6
L' Morecambe Bay	12	57.8	17–190	29	0.10	0.01–0.40	4.9
M Icelandic waters	10	26.1	8–83	54	0.23	0.08–0.67	6.2
N' Anglesey to Solway Firth	124	49.8	4–443	18	0.57	0.26–1.50	10.4
				101	0.60	0.13–1.30	10.9
				79	0.64	0.10–1.40	11.1
				44	0.61	0.21–1.30	11.0
				49	0.05	0.02–0.12	1.9
				11	0.03	0.01–0.07	1.2
				276	0.45	0.03–1.5	9.1

*Only water samples analysed by the technique described by Gardner and Riley⁴ were used to calculate means of mercury concentrations, so as not to incorporate any inter-laboratory bias. Dissolved Hg levels were taken from (1) the January 1974 survey; (2) the previous surveys of the Irish Sea³; (3) the English Channel and Icelandic waters¹⁵; (4) the Mersey Estuary (results to be published) and (5) the Bristol Channel⁵.

†From refs 1 and 2.

‡Within 25 miles of the coast.

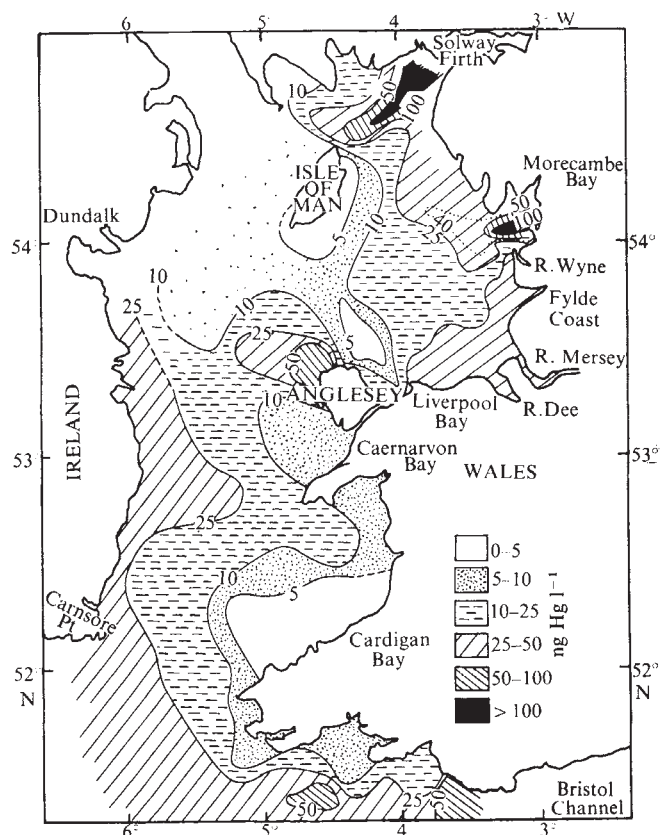
to the south and the north-east of Anglesey, and near the Isle of Man. They were probably caused by rapid removal of dissolved Hg into suspended matter⁵ and sediments disturbed by the rough conditions. Levels of dissolved Hg were high in the Mersey estuary, Liverpool Bay, Morecambe Bay, Solway Firth, north-west Anglesey and the Bristol Channel. These areas are all associated with industries that use and lose Hg, for example chloro-alkali and bromine-producing plants. Plumes of water with high or low levels of dissolved Hg can be traced in an anticlockwise gyre known to circulate between England, the Isle of Man and Anglesey⁷.

The environmental chemistry of Hg is complex. In water, ionic Hg predominates but fish tissue contains on average > 80 % methyl Hg (ref. 2). Dissolved Hg is removed rapidly from water to particulate matter and subsequently to sediments where natural methylation can occur⁸. Fish accumulate Hg and have concentrations in their tissue that are due to variables which include: the availability of food and its Hg content; the chemical form and concentration of dissolved Hg; the fish species and trophic level; and the growth rate, sex and length of the fish^{9, 10}.

Table 1 gives the boundaries of the sample zones, the number of samples, the mean and ranges of Hg concentrations and the concentration factors from water to fish in each zone. The ranges of concentrations of dissolved Hg measured in each area are large. This is because the stations were selected to cover all types of environment—from open oceans to water downstream of an erupting volcano, and from surf zones, which can have undetectable dissolved Hg concentrations, to sewage outfalls and sludge dumping grounds. Therefore confidence intervals of all the data have not been used because they are quite misleading. It must be assumed that the amounts and sources of Hg and the mechanisms for its removal within each boundary were fairly constant during the period of the investigation, and that the sample areas and the number of water samples are sufficiently large that the calculated mean concentrations of dissolved Hg in each compartment represent a homogeneous (numerically mixed) water mass.

Various curves were fitted to the calculated mean concentrations, and the line of best fit (correlation coefficient

0.92), from which point H is excluded, was: $y = 0.013x - 0.179$ (Fig. 2) where $x = \text{Hg concentration in water (ng l}^{-1}\text{)}$, $y = \text{Hg concentration in marketable fish (p.p.m. wet weight)}$. (To determine whether point H was significantly different from the other points making up the regression line, a t test with 11

Fig. 1 Distribution of dissolved Hg in the Irish Sea: January–February 1974 (ng l^{-1}).

degrees of freedom¹¹ was used. The point was significantly different at the 1 % level.)

When conclusions are drawn from this type of mean trend line, several other constraints should be taken into consideration. For example, the fish sampled within each boundary would probably not all be of the same species; nor are the numbers of each species, their size distributions or their trophic level and associated dietary habits^{12,13} likely to be identical. In the Irish Sea the principal fish caught are plaice, whiting, sole, cod, skate, ray and herring. The main cod fishery is between Cumberland and the Isle of Man although cod are caught in Liverpool Bay together with rays. Herring are fished in the northern Irish Sea mainly around the Isle of Man. Shrimps are trawled from along the coasts, and in the estuaries of Lancashire and Cheshire from Morecambe Bay to the River Dee. Plaice and sole spawn off the Great Orme and St Bees Head and occupy nursery grounds in the estuaries of the rivers Dee, Mersey and Ribble and in Morecambe Bay. They disperse throughout the northeast Irish Sea when mature. The chief whiting fishery is off the Irish coast.

It must therefore also be assumed that the samples of marketable fish used here are sufficiently large to give means representative of the long term environmental conditions within each boundary. Comparison of the results reported here with data collected in consecutive years^{1,2} suggests that this is the case.

Figure 2 shows the concentration factors of Hg from water to fish in different areas ranging from 2.9×10^3 in unpolluted distant fishing grounds to 10.6×10^3 within Liverpool and Morecambe bays. A concentration factor of 20.5×10^3 was obtained for Hg from water to fish in the central Irish Sea and around the Isle of Man (H in Fig. 2). This is illogical as it is almost twice that obtained in the polluted inshore regions.

The anomaly is probably explained by the low dissolved Hg levels found throughout the year in zone H. However, the somewhat higher Hg levels observed in fish from zone H could be accounted for if contaminated food particles from the polluted coastal areas were swept into central waters by the residual anticlockwise current which has been reported⁷. Also some of this Hg could have been accumulated when the young fish lived in, or the adult fish occasionally fed in, the shallow polluted inshore nursery grounds¹⁴.

Thus the habits and type of local fish, and the residual currents of the water system must be taken into account when fishing grounds are located 'downstream' of areas which receive industrial effluent. The significance of the fit for so many other data points indicates that even though the aquatic Hg cycle is complex, much 'order' is found.

A limit of 0.5 p.p.m. of Hg in edible fish protein has been set in most countries although some have chosen limits from 0.4 to 1.0 p.p.m.¹⁵. The limited deduction that can be made, from the correlation described here, is that if coastal waters close to industrial centres have high seasonal mean dissolved Hg concentrations, there is a higher probability of finding fish in those waters that will contain high Hg levels, and, these fish should be more subject to scrutiny than fish caught in an area with low dissolved concentrations of Hg.

It might tentatively be suggested that suspect fish should be sold only as fish meal. Hg concentrations in fish processed in this way are diluted by uncontaminated fish before sale or by other feedstuffs in the diet. In the Irish Sea the suspect fishing grounds E, F, L, M and O extend along the coast from Anglesey to St Bee's Head and include all the estuaries within that boundary.

The effects of Hg pollution on fish are greater close to the source. Local fisheries landing their catches in Fleetwood, Whitehaven and Maryport work most of the time in the Liverpool Bay and Morecambe Bay grounds. Since only 3 % of the total catch comes from the Irish Sea, the normal intake of Hg from fish in the diet of the average consumer in Britain should not be a hazard².

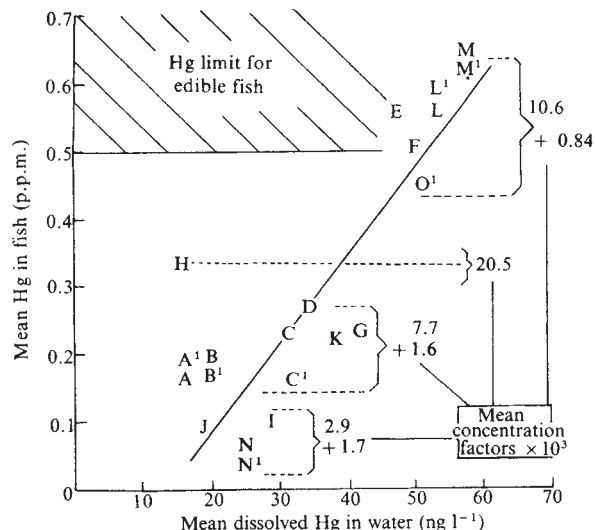


Fig. 2 Correlation between mean Hg content of edible fish caught in various fishing grounds worked from the United Kingdom and mean Hg concentrations of water samples collected in and around the same localities (omitting the central Irish Sea waters H from the regression curve).

Even after Hg is no longer released into the marine environment the levels in fish in certain areas apparently continue to increase before stabilising¹⁶. Sediment samples taken off the Fylde coast and off the Mersey estuary contained 130–380 ng g⁻¹ compared with 10–30 ng g⁻¹ for the rest of the Irish Sea (P. D. Jones, personal communication). It is likely that this effect could cause high Hg levels in fish from these fishing grounds for a long time. Therefore a continuing monitoring programme should be undertaken in the contaminated areas of Liverpool and Morecambe bays. This is in agreement with the recommendations of the Pharmacology Subcommittee of the Committee on Medical Aspects of Food Policy².

I thank the captain and officers of the RV Edward Forbes for their help, P. D. Jones for assistance with the sample programme, Professor J. P. Riley for arranging finance, and R. Sandland for helpful comments. I was supported by NERC (grant GR/3/1498).

D. GARDNER*

Department of Oceanography,
University of Liverpool, UK

Received 6 December 1976; accepted 13 January 1978.

*Present address: CSIRO Division of Fisheries and Oceanography, PO Box 21, Cronulla, New South Wales 2230, Australia.

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Deaths of owls traced to insecticide-treated timber

THE Zoological Society of London has a large collection of owls (Strigiformes) at Regent's Park. Usually, between 50 and 70 individuals of 25 species can be seen in the collection at one time. Between March 1974 and September 1976, 55 owls of 21 species died. This was an unusually large number, because these species present relatively few management problems. We now report that a significant proportion of the dead owls contained dieldrin residue, which could be traced back to the mice on which they were fed, and in turn to the sawdust used as bedding for the mice.

Seventeen of the deaths, almost all occurring during the first half of the period, were initially considered to have been due to debility associated with senility; the birds had been in the collection between 8 and 22 yr. In 10 further cases, postmortem findings other than poisoning could have contributed significantly to the death of the birds. The remaining 28 carcasses were in good condition and no significant abnormalities were initially detected. Of the last group, 20 died during the first 4 months of 1976, 15 of these in April. Postmortem findings in these 28 birds were limited to congested livers and kidneys. In seven cases there were also congested meningeal membranes or subarachnoid petechial haemorrhages. No significant bacteria, viruses or parasites were found and no abnormalities were seen in the major organs on histopathological examination. Of these 28 birds, 19 were found dead; the remaining nine were found collapsed, and suffered periodic convulsions, particularly when handled. They died within 1–6 h of being discovered.

At this stage, the presence of a toxic substance in the owls was suspected. There had been no recent changes in the management of the aviaries and no potential poisons are used near the birds. The food was thought to be a possible source.

The owl collection is kept in four groups. Of the 28 unexplained deaths, 24 occurred in two of the groups consisting mainly of small species fed almost exclusively on laboratory mice. Some of the larger species also succumbed, but all these birds were found to be in poor condition. The larger owls are fed principally on rats, although mice are occasionally included in their diets. Increased losses among other bird species fed mainly on mice at the zoo were not noticed during this period.

Livers from four of the owls were examined for the presence of heavy metals and only clinically insignificant traces were found. Two further livers, examined for the presence of organochlorine insecticides, yielded dieldrin. In confirmation of this finding, and in a search for the source of the dieldrin, livers from 22 of the 55 owls and brains from 10 were examined. These samples, together with mouse tissues, the mouse diet, and bedding

materials for both owls and mice, were analysed for organochlorine insecticide residues by gas-liquid chromatography (GLC) with electron-capture detection. The results are given in Table 1. The residue present in each category of sample was confirmed qualitatively and quantitatively by GLC/mass fragmentography. At least three of the *m/e* values 380, 345, 277 and 263 were monitored in each case.

Apart from the two lowest dieldrin values, in the livers of a great eagle owl (*Bubo bubo*) ($1.7 \mu\text{g g}^{-1}$) and an Indian eagle owl (*Bubo bubo bengalensis*) ($7.8 \mu\text{g g}^{-1}$), the mean concentrations of dieldrin in owl liver and brain were similar to those found in quail and pigeon which died as a result of dieldrin feeding¹.

With the exception of the two eagle owls, the geometric mean dieldrin concentration in owl liver was $29 \mu\text{g g}^{-1}$ (range 13–46) and in brain $15 \mu\text{g g}^{-1}$ (range 11–25). In quail, the mean concentration in liver was $40 \mu\text{g g}^{-1}$ (range 35–46) and in brain $17 \mu\text{g g}^{-1}$ (range 16–19). The corresponding figures for pigeon were $46 \mu\text{g g}^{-1}$ (range 38–56) and $20 \mu\text{g g}^{-1}$ (range 18–22). The ratio of the concentrations in liver to those in brain was 1.9 for owls (paired samples only), for quail 2.3 and for pigeon 2.2. Liver concentrations of $10 \mu\text{g g}^{-1}$ or more were considered to be lethal in wild kestrels (*Falco tinnunculus*) and barn owls (*Tyto alba*)². Examination of the chromatograms did not reveal the presence of any other organochlorine compound at a concentration significant in the context of the owl deaths. This suggested that dieldrin was the sole or major contributory cause of death in at least 20 of the owls.

The ratio of dieldrin to *pp'*DDE concentrations in owl liver greatly exceeded that found in wild birds, and this finding, with the absence of aldrin, suggested that the owls had ingested the dieldrin during their stay at the zoo. The concentrations of dieldrin in the mice were thought to be sufficient to account for the residues present in the owls. Analysis of further samples of mice showed that the contamination was a continuing one.

The laboratory rodent diet contained concentrations of dieldrin insufficient to produce those found in the mice. The sawdust used by the mouse supplier as bedding contained a high concentration of dieldrin, and had been obtained from a builder who was using a timber preservative containing dieldrin to protect against woodworm.

The case of two eastern screech owls (*Otus asio*) seems to be anomalous, in that these birds were not fed at the zoo. They were illegally imported into Britain, confiscated by the UK Customs, and deposited at the zoo, where they died within three days in a debilitated condition, not having fed. Despite the entirely different history of these two birds, the dieldrin content of their livers (41 and $26 \mu\text{g g}^{-1}$) was within the range of those kept at the zoo over a long period. In their case, the evidence points to a source of dieldrin other than the mice being fed to the owls at the zoo.

The evidence presented here therefore suggests that the dieldrin residue present in the sawdust was sufficient to have caused the concentrations found in the mouse colony, and sufficient therefore to be the source of dieldrin in the owl collection at the London Zoo.

We thank Miss C. Carroll for her assistance, Mr S. Pugsley for the postmortem examination and Miss C. Hall for help with the analyses. Dr I. Keymer carried out some of the earlier postmortem examinations.

D. M. JONES

Zoological Society of London,
Regent's Park, London NW1, UK

D. BENNETT
K. E. ELGAR

Shell Research Ltd,
Shell Toxicology Laboratory (Tunstall),
Sittingbourne, Kent, UK

Received 1 November 1977; accepted 17 January 1978.

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Table 1 Concentrations of dieldrin found in various samples

Sample	No. of samples	Dieldrin concentration ($\mu\text{g g}^{-1}$)	
		Geometric mean	Range
Owl liver ex-zoo	22	24	1.7–46
Owl liver, 10 paired samples	10	28	20–44
Owl brain, 10 paired samples	10	15	11–25
Mouse ex-supplier	1	0.6	—
Mouse liver ex-supplier	1	0.5	—
Mouse liver 'old colony' ex-supplier	4	3.1	2.3–5.6
Mouse liver 'new colony' ex-supplier	4	2.1	0.9–5.2
Rodent diet ex-supplier	9	0.007	0.002–0.01
Sawdust bedding for mice ex-zoo	1	<0.05	—
Peat bedding for owls ex-zoo	1	0.02	—
Sawdust bedding for mice ex-supplier*	1	94	—

*Several samples were subsequently analysed by the supplier. Their dieldrin contents ranged from several tens to several hundreds of micrograms per gram.

Lid closure and refractive error in macaque monkeys

It has been suggested that lid suture in visually immature macaques¹ and tree shrews² causes myopia. Wiesel and Raviola suggested that this myopia is of the axial type and caused by elongation of the globe¹. Sherman *et al.* were unable to correlate the axial length with the degree of myopia in their experimental animals and suggest that lenticular or corneal anomalies may account for myopia in lid-sutured eyes². If myopia could thus be produced experimentally in one eye, the other eye serving as a control, a useful and urgently needed animal model for the study of the pathogenesis of myopia and of the effects of anisometropia on development of visual functions would be available. However, we have been unable to confirm a consistent relationship between myopia and lid suture in the macaque. In view of this discrepancy we are reporting the refraction data collected from 13 male colony-bred rhesus monkeys (*Macaca mulatta*) that were used for studies of the behavioural, neurophysiological and anatomical effects of lid suture on the development of visual functions³⁻⁸.

The technique of lid closure differed slightly from that used by Wiesel and Raviola¹ who excised the margins of both lids and portions of the palpebral conjunctiva followed by suturing of the lids with silk mattress sutures. Sherman *et al.* sutured the lids in a similar fashion². As it could be argued that the type of lid suture used may have some bearing on the difference in results, we report our procedure in detail. After anaesthesia of the animal with an intramuscular injection of ketamine hydrochloride (Ketalar) and infiltration of the upper and lower lids with 2% proparacaine, the upper and lower lids were split

in the grey line and the upper and lower tarsus were dissected from the skin by blunt dissection. Three double armed 6-0 silk sutures were passed through the upper margin of the lower tarsus in its nasal, medial and temporal aspects, threaded between the skin and upper tarsus, and brought out through the skin below the eyebrow. By tying the sutures over silicone pegs, the lower tarsus was thus pulled between the skin and the tarsus of the upper lid. The lash line and the underlying skin were then excised from the upper and lower lid margins and the wound closed with an intercuticular 5-0 plain catgut suture. The silk sutures usually fell out spontaneously after 10 d at which time the lids had firmly fused. The lower tarsus retracted into its normal position after 2 to 3 weeks so that the eye was covered by only a thin layer of skin, reducing the amount of light entering the pupil by 90% (ref. 9).

This surgical technique provides a secure lid closure that withstands the most vigorous attempts by the mother animal to open the sutured lid by licking the wounds or pulling the sutures with her teeth.

After varying periods of lid closure, and after infiltration of the lids with 2% proparacaine, the lid was opened by making a small incision into the fused lateral aspects of the inferior palpebral fissure and introducing one blade of Steven's scissors between cornea and skin and opening the fused interpalpebral fissure by closing the scissor blades. If excessive bleeding was encountered, the upper and lower tarsal plates were united with the lid margins with a running 5-0 plain catgut suture.

In most animals, the other eye was then closed with the same technique after which behavioural data on the visual deficit induced by lid closure during visual immaturity were gathered. Refraction by retinoscopy, using a Copeland Streak retinoscope and loose lenses from a trial case was performed either immediately, after opening the lid, or several months later and before the neurophysiological experiment. Cycloplegia was obtained with several conjunctival applications of 1% atropine sulphate. The retinoscopy data were obtained by two different observers, one a clinically experienced ophthalmologist (G.Kv.N.) and the difference between the two observations was negligible.

The data are presented in Table 1 grouped according to the age at which the lids were closed and the duration of the lid closure. In animals numbered 1-11, lid closure was performed in one eye within the first year of life. Disregarding the eyes in which the lids were fused after the age of 12 months, and assuming that at least 2 weeks duration of neonatal lid closure was required to produce myopia, the data reveal that the eye closed during the first year of life was more myopic only in monkeys 3, 6 and 8, whereas that eye was hypermetropic in monkeys 4, 7 and 9. There was less myopia in the neonatally sutured eye than in the other eye, closed after the age of 12 months in monkey 11 and no difference in the refractive error existed between the two eyes in monkey 10. If lid closure was performed in monkeys older than 12 months and the lids remained closed for periods ranging from 4 weeks to 72 months, myopia was found in all but one monkey³ but was not always more pronounced in the sutured eye.

Of special interest is monkey 5 in which both eyes were closed at the age of 2 weeks and opened at the age of 3 months. If lid closure produces myopia, one would certainly expect a similar refractive error in both eyes after this experiment. However, the right eye was found to be myopic and the left eye hypermetropic. This finding is in contrast with a similar experiment described by Wiesel and Raviola, who closed both eyes of a monkey at the age of 5 weeks and found -11.00 D myopia in both eyes 11 months later.

Even though the high incidence of anisometropia is at variance with the distribution of refractive errors reported in normal monkeys¹⁰ our data show a preponderance of myopia which is in accord with the previously noted tendency for colony-bred monkeys which are raised in cages to have myopic refractive errors¹⁰. It is beyond the scope of this report to discuss whether this is caused by genetic or environmental

Table 1 Lid closure and refractive error in macaque monkeys

No.	Identification	Age at closure	Duration	Refractive error
1	403	OD: 1 wk OS: no closure	1 wk	- 4.00 sph + 0.50 sph
2	N 8	OD: 4 wk OS: no closure	1 wk	+ 4.50 sph + 5.75 sph
3	L 10M	OD: 4 wk OS: 13 mth	2 wk 12 mth	- 4.00 sph + 0.50 sph
4	F 284K	OD: 4 wk OS: 16 mth	4 wk 30 mth	+ 4.00 sph - 3.00 sph
5	L 35M	OD: 2 wk OS: 2 wk	3 mth 3 mth	- 1.00 sph + 2.50 sph
6	7254	OD: 9 wk OS: 8 mth	8 mth 28 mth	-13.75 sph -10.25 sph
7	D 21J	OD: 7 wk OS: 14 mth	14 mth 57 mth	+ 2.00 sph - 3.00 sph
8	E 291	OD: 1 wk OS: 20 mth	20 mth 21 mth	-12.00 sph - 5.75 sph
9	B 43	OD: 2 wk OS: 27 mth	24 mth 72 mth	+ 3.00 sph - 2.00 sph
10	B 35A	OD: 1 wk OS: 20 mth	26 mth 5 mth	- 4.00 sph - 4.00 sph
11	E 121J	OD: 16 mth OS: 12 mth	17 mth 4 mth	- 3.50 sph -0.50+1.00 cyl axis 180
12	E 293	OD: 20 mth OS: no closure	38 mth	-7.50+1.50 cyl axis 180 + 0.50 sph
13	E 291H	OD: no closure OS: 24 mth	30 mth	- 1.50 sph - 1.75 sph

OD, right eye; OS, left eye; wk, week; mth, month.

factors. However, we cannot confirm the consistent relationship between neonatal lid fusion and the quantity of myopia reported by two other laboratories and are unable to explain this discrepancy.

It is remotely possible that the technique of lid closure may have an influence on the refractive error since an extremely tight closure caused by sacrificing a considerable portion of the upper and lower lid margins could conceivably prevent growth of the eye in an anterior direction and cause expansion of the retroequatorial aspects of the globe and thus its elongation. This possibility is certainly worthwhile exploring experimentally. Also, it seems important to perform refractions of the eyes not only after but also prior to neonatal lid fusion to support the argument of a causal relationship between lid fusion and the development of myopia.

This work was supported by grant EY 01120 from the US NIH, Department of Health, Education and Welfare.

GUNTER K. VON NOORDEN*
M. L. J. CRAWFORD

Department of Ophthalmology,
Baylor College of Medicine, and
The University of Texas
Graduate School of Biomedical Sciences,
Houston, Texas 77030

Received 4 October; accepted 21 December 1977.

*To whom reprint requests should be addressed: Department of Ophthalmology, Texas Children's Hospital, PO Box 20269, Houston, Texas 77025.

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Mitogenic stimulation of the host enhances susceptibility to scrapie

THERE are several reasons for concluding that scrapie agents depend, for extraneural replication, on some components of the host's lymphoreticular system being functional¹. Agent replication occurs earliest in the spleen and lymph nodes² and some factors which make an animal more susceptible to infection by conventional viruses have the opposite effect with scrapie. For example, infection with scrapie by the intraperitoneal (i.p.) route is more difficult in neonatal than in adult mice³, in mice treated with a large dose of cortical steroids⁴ and in mice with severe malnutrition (A.G.D., unpublished). These findings raise the question whether the opposite effect can be produced, so that mice given treatments which stimulate any sector of the immune system would be infected more readily with scrapie agent given extraneurally. The results presented here confirm this expectation in mice treated with the mitogen phytohaemagglutinin (PHA), which increases the activity of lymphoreticular cells.

The C3H/FaDk mice used were 4–12 weeks old at injection, with experimental subgroups having similar age distributions. The ME7 scrapie agent was used as a 10^{-4} saline dilution of brain homogenate (2,000g, 15 min supernatant) from a C57BL mouse: the i.p. volume was 0.02 ml. This dose, although containing 10^2 intracerebral LD₅₀ units, was chosen to be less than the smallest infective i.p. dose for untreated C3H mice and it was approximately five times more dilute than the normal i.p. LD₅₀ endpoint. In the first two experiments PHA (Burroughs-Wellcome reagent grade: 5 ml-dried, dissolved in 1 ml of saline solution) was given i.p. as a single 0.02 ml injection at various intervals before or after the scrapie injection or, in one group, the PHA was mixed with an equal volume of the scrapie inoculum and 0.04 ml of the mixture was injected.

Table 1 Incidence of scrapie and mean incubation period (days $\pm$ s.e.m.) in C3H mice injected i.p. with <1 i.p. LD₅₀ unit of ME7 agent. The mice were also injected i.p. with PHA at various intervals

Interval between PHA dose and scrapie dose (h)	Scrapie incidence	Incubation period
–24	0/8	—
–3	6/7	322 $\pm$ 13
–1	6/7	323 $\pm$ 14
0	6/8	339 $\pm$ 7
+1	0/7	—
+3	0/6	—
+24	0/8	—
No PHA	0/8	—

The aim of the first experiment was to determine whether it was possible to infect mice, which were stimulated by a mitogen, with a dose of scrapie agent which would otherwise not be infective by the i.p. route. The results show that scrapie only occurred in mice which received PHA and, even then, only in mice given the PHA at the time of injection with ME7 agent or up to 3 h beforehand (Table 1). It is estimated from the incubation periods that the PHA given 0 to 3 h before the ME7 agent increased its operational titre by between 0.5 and 1.5 $-\log_{10}$ i.p. LD₅₀ units.

This experiment did not exclude the possibility that incubation might be shortened nonspecifically by any i.p. injection shortly before injecting scrapie agent. A further experiment, therefore, compared mice injected i.p. with isotonic saline or with PHA 3 h before they were injected with agent: the 10^{-4} inoculum used had about five times more scrapie infectivity than in the first experiment and some cases occurred in the groups not given PHA, but at lower incidence and at significantly longer incubation (Table 2). The consequence of using this infective dose was to shift the PHA effect largely from a difference in incidence to a difference in incubation period. The results indicate that any nonspecific effect is small compared with the PHA effect.

Additional confirmation of the basic finding was provided by a serial i.p. titration of ME7 in C3H mice. Two sets of dilutions were made, one using isotonic saline as the diluent, the other using the PHA solution: the respective Reed–Muench titre estimates were 3.8 and 5.2 $-\log_{10}$ i.p. LD₅₀ units. This PHA treatment had, therefore, raised the effective i.p. titre to only 1 log unit less than the intracerebral titre.

The increased susceptibility produced by PHA could involve two distinct processes: increased access of agent to cells involved in its replication and/or impaired degradation (or excretion) of infectivity. Even though scrapie is a 'slow infection', a striking feature is the importance of precise timing of the initial events in this system, with the mixed inoculum group in the first experiment giving quite different results from those given PHA only 1 h later. It would be premature to attempt to single out one or more types of PHA-responsive cell involved in the primary events in scrapie infection by collating these results with the finding^{5,6} that thymic lymphocytes are not essential to

Table 2 Incidence of scrapie and mean incubation period (days $\pm$ s.e.m.) in C3H mice injected i.p. with 1 i.p. LD₅₀ of ME7 agent

Treatment 3 h before injection with scrapie	Scrapie incidence	Incubation period
PHA i.p.	6/7	307 $\pm$ 11*
Saline i.p.	4/6	357 $\pm$ 7
None	2/6	352 $\pm$ 4†

* Probability of difference in incubation period compared with i.p. saline treatment, $P < 0.01$; compared with no treatment, $P < 0.01$.

† Probability of difference in incubation period compared with i.p. saline, $P > 0.2$.

scrapie pathogenesis. Indeed, there is recent evidence that mitogens may exert their effects on lymphocytes via macrophages⁷, which emphasises that there is a widening range of cell types which will have to be considered for scrapie pathogenesis. We also have preliminary data showing that the bacterial mitogen, lipopolysaccharide, has broadly the same effect as PHA on scrapie infectivity, which is important because this mitogen preferentially affects a different range of cells from PHA⁸.

As mitogens are widely present in the environment, either in food or arising from infection with various microorganisms, these findings for scrapie could have important epidemiological implications for the sheep disease and for the analogous human infections, Creutzfeldt-Jakob disease and kuru. It seems reasonable to expect that the present type of result will also apply to these infectious agents entering, say, orally or through skin abrasions, and that mitogens could increase susceptibility to natural infection by very low doses of scrapie and related agents.

Since submitting this paper we have learnt of the observation of a shortening of scrapie incubation in mice treated with methanol extract of BCG⁹ which may have the same basis as the PHA effect.

A. G. DICKINSON
H. FRASER
IRENE MCCONNELL
G. W. OUTRAM

A.R.C. Animal Breeding Research Organisation,
West Mains Road, Edinburgh,
and
Moredun Research Institute,
Gilmerton Road, Edinburgh, UK

Received 25 October 1977; accepted 13 January 1978.

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Antileishmanial activity of antimonials entrapped in liposomes

THE use of liposomes in chemotherapy as a method of ensuring that drugs reach the diseased or defective tissues suffers from the drawback that, on intravenous injection, the bulk of the material is rapidly cleared by the phagocytic cells of the liver¹, and does not reach the desired target. We report here a system in which advantage is taken of this behaviour to treat visceral leishmaniasis in mice, in which the amastigote parasites reside unharmed in vacuoles inside the Kupffer cells of the liver².

Liposomes were prepared from lecithin (purified from egg yolk by the method of Pangborn³) by ultrasonication as described by Bangham and Horne⁴. Lecithin (200 mg) was dispersed in 10 ml fluid, and sonicated in the cold for 5 min. For liposomes containing drug, 2 g either of antimony potassium tartrate or sodium antimony gluconate were included in the dispersion. Otherwise, the material was dispersed in distilled water alone. After sonication, the suspension was passed through a glass fibre filter pad, dialysed for 3 d against 2 l of normal saline (with three changes of medium) and then concentrated 10-fold using an Amicon XM100 filter. The quantity of antimonial drug retained in the concentrate was measured by atomic absorption spectroscopy. The filter held back up to 90% of the antimony contained in the dialysate. This material was found to sediment within 30 min at 100,000g. The above procedure gave liposomes containing typically a total of 5 mg drug. After storing for 1 week at room temperature, at least



Fig. 1 Electron micrograph of section of mouse liver showing liposome containing potassium antimony tartrate 1 h after intravenous injection. Tissue fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer post-fixed in 1% osmium tetroxide and embedded in Araldite. ($\times 200,000$).

50% of the antimony was retained within sedimentable liposomes, the remainder having escaped into solution. A fresh preparation was used for each experiment described. Figure 1 shows an example of an antimony-containing liposome which displays the characteristic multilamellar structure. Preliminary observation by X-ray microanalysis has provided additional evidence for the presence of antimony in these vehicles.

The effect of drugs on a leishmanial infection was assayed by a modification of the method of Stauber *et al.*⁵ using the mouse model of Bradley⁶. The concentration of drug was adjusted to 1 mg ml⁻¹, and injected intravenously into NMR1 mice (approximate weight 20 g) which had been inoculated intravenously 10 d previously with amastigotes of an Ethiopian isolate of *Leishmania donovani* s.l. (isolate no. HU3, Liverpool designation LV9). This parasite is maintained routinely by passage through hamsters and transferred to mice in the form of a spleen cell suspension. The mice were killed 2 d after injection of the drug, and the extent of the infection assayed microscopically by counting the number of amastigotes per 200 liver cell nuclei in an impression smear.

Table 1 shows that drug incorporated into liposomes had a much greater effect in reducing the parasite count in the liver than equivalent quantities of either liposomes or drug injected alone. Tartrate, 0.4 mg per animal (20 mg per kg body weight), given over a period of 3 d, was sufficient to abolish the infection completely as judged by light microscopy (Expt 1). An identical result was obtained when the same quantity was administered in a single dose (Expt 2). A significant reduction in the number of parasites was also observed after administration of a liposome suspension in which the tartrate had been dissolved at a concentration of 0.8 mg per ml just before injection. This result suggests that there may be some uptake of the drug by lecithin liposomes from the aqueous medium. Over the range of doses tested, there is a linear response of probit activity against log dose (Fig. 2). In addition, Table 1 shows that the enhanced activity exhibited by tartrate in the liposomes was also given by the drug sodium antimony gluconate, which is formulated for clinical use under the trade name Pentostam (Burroughs Wellcome).

The ideal drug carrier has three important properties. First, it must protect the drug from host metabolic processes and, vice versa, protect the non-target host tissues during the time taken for the drug to reach the target. Second, it must transport the drug directly to the desired tissue, and, finally, it may enhance the uptake of the drug by the cells of the target tissue,

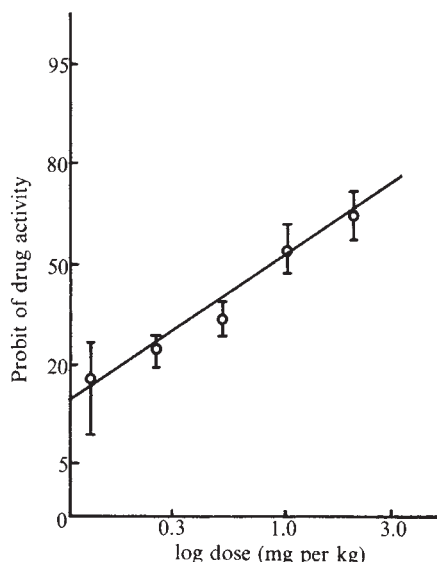


Fig. 2 Relation between probit of activity and dose of potassium antimony tartrate, using data from Table 1 (Expt 3).

and perhaps increase its efficacy within the defective cell.

In the experimental model described here, antimony-filled liposomes seem to satisfy most of these requirements. Antimony potassium tartrate was one of the first and most potent agents used for curing leishmaniasis,⁷ but effective treatment necessitated prolonged exposure to highly toxic levels of drug. In our system, levels close to the lethal dose of 50 mg per kg were without effect; when entrapped in liposomes, however, a single dose of 20 mg per kg (Expt 2) was completely effective in eliminating the infection. Measurement of the concentration of antimony in plasma samples after injection of tartrate within liposomes showed that the material was cleared from the blood with a half life of approximately 1 h. At this time, antimony-containing liposomes were found in

the liver (Fig. 1). In contrast, the free drug was cleared rapidly from the plasma, and was not detectable by our method 15 min after intravenous administration. Lippincott *et al.*⁸ have shown that free tartrate is taken up by erythrocytes during this time. Also, other workers have shown that liposomes are taken up by all organs encompassing the mononuclear phagocyte system (MPS)⁹, and that uptake of therapeutic drugs into lysosomes of phagocytic cells can be enhanced *in vitro*¹⁰ and *in vivo*¹¹ by incorporation of the drug into liposomes.

In view of the fact that liposomes and leishmanial parasites share a propensity for cells of the MPS, it is reasonable to hope that targeting of antimonial drugs at present in use, by entrapment within liposomes, may be feasible in a range of clinical manifestations of the disease, and especially where amastigotes reside within macrophages of the liver, spleen, bone marrow and lymphatic system.

We thank Mr G. Moore for technical assistance, and Dr C. Green for helpful discussion. Financial support was provided by the Wellcome Trust, WHO and the US Army Research and Development Command (Contract DA-ERO-124-74-G0049).

R. R. C. NEW

Department of Biochemistry,
University of Liverpool,
PO Box 147, Liverpool, UK

M. L. CHANCE
S. C. THOMAS
W. PETERS

Department of Parasitology,
Liverpool School of Tropical Medicine,
Pembroke Place, Liverpool, UK

Received 20 October 1977; accepted 16 January 1978.

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Table 1 Survival of amastigotes in the liver after treatment with drug-loaded liposomes

Expt	Drug	Treatment	Dose administered (mg per kg body weight)	Parasites per 200 cell nuclei $\pm$ s.e.
1	Potassium antimony tartrate	None	None	240 $\pm$ 50
		Drug alone	20	260 $\pm$ 17
2	Potassium antimony tartrate	*Drug within liposomes	20	0
		None	None	849 $\pm$ 86
		Liposomes alone	None	863 $\pm$ 81
		Drug alone	20	760 $\pm$ 63
		†Drug + liposomes	20	304 $\pm$ 66
3	Potassium antimony tartrate	*Drug within liposomes	20	0
		None	None	297 $\pm$ 16
		*Drug within liposomes	0.125	247 $\pm$ 29
			0.25	225 $\pm$ 17
			0.5	199 $\pm$ 15
			1.0	113 $\pm$ 30
4	Potassium antimony tartrate	None	None	508 $\pm$ 86
		Drug alone	10	513 $\pm$ 34
		*Drug within liposomes	10	46 $\pm$ 19
	Sodium antimony gluconate		5	86 $\pm$ 21
		Drug alone	10	638 $\pm$ 77
		*Drug within liposomes	10	63 $\pm$ 15
			5	172 $\pm$ 29

Groups of six NMR1 mice were inoculated with amastigotes of *L. donovani* on day 0, and received their first dose of drug on day 10. In Expt 1, the drug was administered over 3 d (days 10, 11, 12) and the animals were killed on day 14. In Expts 2, 3 and 4, the drug was given as a single dose, and the livers taken for assay on day 12.

*Drug incorporated into liposomes during sonication. 1 mg of drug is contained in approximately 40 mg phospholipid.

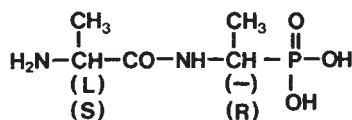
†Drug mixed with a pure liposome suspension 10 min before injection.

Phosphono-peptides, a new class of synthetic antibacterial agents

ANTIBIOTICS of the group that includes the penicillins, the cephalosporins, D-cycloserine and vancomycin, act by interfering with the biosynthesis of bacterial cell walls^{1,2}. This is achieved by inhibiting the formation, or the subsequent utilisation in cross-linking, of the terminal D-Ala-D-Ala units which occur in the peptide chains of the cell-wall peptidoglycans of all pathogenic bacteria so far investigated³⁻⁵. We have sought new synthetic compounds, related to D-Ala-D-Ala, that have similar antibacterial action. We report here that certain phosphono-peptides, of which L-alanyl-L'-l-aminoethylphosphonic acid (alaphosphin) is typical, inhibit the growth of various important pathogenic bacteria *in vitro* and in infected animals.

In 1971, we initiated investigations aimed at identifying synthetic peptide mimetics related to D-Ala-D-Ala that might have antibacterial properties. The C-terminal alanine residue was replaced by a related unit with variations in stereochemistry and in the sidechain; the carboxyl group was replaced by functions that included: tetrazolyl⁶, sulphonyl⁷, sulphonamido-, phosphoryl⁸, substituted phosphoryl- and methylphosphinyl. More than 300 di- to pentapeptide mimetics were prepared by combining false C-terminal aminoacids with various L- and D- α -amino carboxylic acids. The phosphoryl series, in particular, compounds containing residues of L-l-aminoethylphosphonic acid, L-Ala(P), had the most interesting antibacterial properties (see Tables 1 and 2). The dipeptide mimetic I, which we have named alaphosphin, has been chosen

as a typical representative of the series, although related compounds have different antibacterial spectra and might be preferable for particular applications in therapy. (The stereochemistry of L-aminoethylphosphonic acid has been elucidated by X-ray diffraction studies (J. J. Daly, unpublished)).



I (alaphosphin)

The antibacterial spectrum of alaphosphin *in vitro* is shown in Table 2. Unlike typical β -lactam antibiotics, alaphosphin is more effective against Gram -ve than Gram +ve organisms. It is more active against *Escherichia coli* (median MIC 0.03 $\mu\text{g ml}^{-1}$, 81 isolates) than antibiotics commonly used in therapy and inhibits the growth of most strains resistant to agents such as ampicillin. Good levels of activity were found for the Gram -ve organisms *Klebsiella aerogenes* (median MIC 1 $\mu\text{g ml}^{-1}$, 41 isolates), *Enterobacter* (median MIC 1 $\mu\text{g ml}^{-1}$, 41 isolates), *Salmonella* (median MIC 4 $\mu\text{g ml}^{-1}$, 26 isolates) and *Serratia* species (median MIC 4 $\mu\text{g ml}^{-1}$, 14 isolates). Some Gram +ve organisms are sensitive, for example *Streptococcus faecalis* (median MIC 1 $\mu\text{g ml}^{-1}$, 41 isolates), *Staphylococcus aureus* (median MIC 4 $\mu\text{g ml}^{-1}$, 39 isolates) but, generally less so than β -lactam antibiotics in common use.

In vivo studies reflect the *in vitro* results. In typical experiments, septicaemia in mice was controlled by the drug introduced at intervals of 1, 3 and 5 h (CD_{50} values for subcutaneous treatment: *E. coli*, 5–10 mg per kg body weight; *K. aerogenes*, 35–350 mg per kg; compare ampicillin values: 0.5–1.0 mg per kg and 80 to > 400 mg per kg respectively). Parenteral administration to other species (rats, dogs, baboons and human volunteers) established that there was rapid absorption and dose-related peak plasma levels were achieved within 20 min. The half life of the drug varied with species from 10 min (mouse) to approximately 1 h (man and baboon). Alaphosphin is absorbed reasonably well by the oral route. Plasma levels for a single 500 mg oral dose in human volunteers were similar to those achieved with 200 mg by intramuscular injection.

Extensive toxicological studies in rodents and baboons suggest that there is a very considerable margin of safety at doses expected to be effective therapeutically in man.

Early studies with alaphosphin established that sensitive organisms developed aberrant morphological forms similar to those observed with cell-wall biosynthesis inhibitors such as D-cycloserine, penicillins and cephalosporins^{9–11}. Several points of evidence indicated that L-Ala(P) was the active agent within the bacterial cell although this amino acid, alone, in the culture medium did not inhibit growth of bacteria significantly; it had to be combined in a suitable di- or higher peptide mimetic to enable transport into the cell by peptide permeases¹².

E. coli cells incubated in the presence of low concentrations of alaphosphin, ^{14}C -labelled in the L-Ala(P) residue, accumulated radioactivity rapidly and lysed within 20–30 min. Electro-

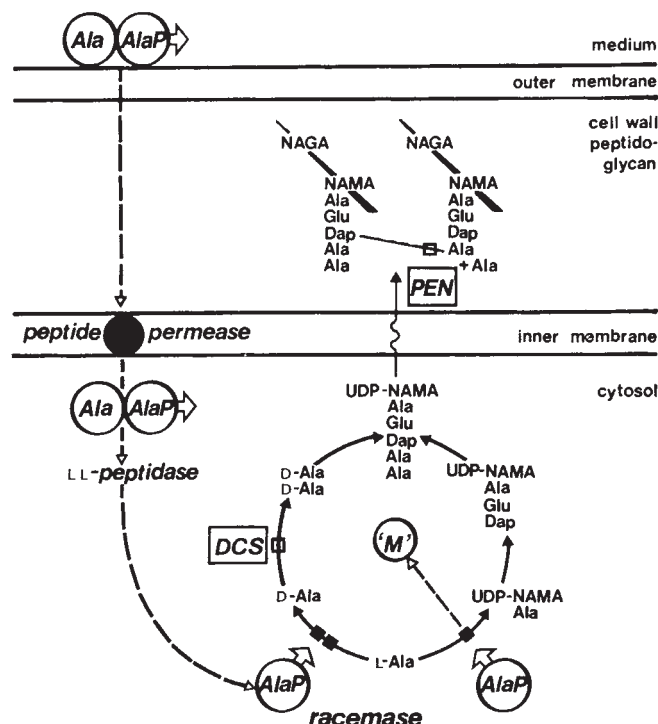


Fig. 1 Mechanism of action of alaphosphin in *E. coli*. AlaAlaP, alaphosphin; DCS, D-cycloserine; PEN, penicillin; UDP-NAMA, uridine diphospho-N-acetylmuramic acid; NAGA, N-acetylglucosamine; M, metabolite of Ala(P).

phoresis of cell contents at intervals during the experiment established that radioactivity was in three fractions: alaphosphin, a trace; ^{14}C -L-Ala(P), the largest part; and some UDP-N-acetylmuramyl- ^{14}C -L-Ala(P) [compound M]. Evidently, incorporation of L-Ala(P) into compound M prevented the formation of the extended peptide chain.

Staphylococcus aureus grown in the presence of alaphosphin accumulated UDP-N-acetylmuramyl-L-Ala-D-Glu-L-Lys rather than the normal pentapeptide with two additional D-Ala residues^{2,4}. This, taken with evidence that L-Ala(P) is a primary inhibitor of L-alanine racemase, isolated from *E. coli* or *Strep. faecalis*¹³, and that no D-Ala(P) was formed by the action of this enzyme, suggested that L-Ala(P) was inhibiting the biosynthesis of D-Ala-D-Ala within the bacterial cell.

The indication from the ^{14}C -incorporation experiments of active energy-linked transport by an L,L-stereospecific peptide permease was supported by evidence that when L,L-alanylalanine was introduced into the culture medium it competed with alaphosphin for the same active transport system.

Figure 1 sets out the pathway of cell-wall biosynthesis for *E. coli*, schematically, the points of inhibition of alaphosphin, penicillins and D-cycloserine are indicated. Since these differ, it

Table 1 Antibacterial activity for mixed stereochemistry phosphono-peptides [(Ala)_n-Ala(P)] compared with alaphosphin

Organism	Alaphosphin		Tripeptides						Tetrapeptides						Pentapeptide			
	LL	LLL	DLL	LLD	LDL	DLD	LDD	LLLL	DLLL	LDLL	LLDD	LDLL	LLDL	LDLD	LDLD	DLDD	DLDD	DLDD
<i>E. coli</i>	0.25	0.25	i	i	i	i	i	0.1	4	256	i	i	i	i	i	i	i	4
<i>K. aerogenes</i>	0.25	0.12	i	i	i	i	i	0.5	32	i	i	i	i	i	i	i	i	16
<i>S. aureus</i>	16	32	i	i	i	i	i	128	i	i	i	i	i	i	i	i	i	i
<i>Str. faecalis</i>	2	0.06	128	32	i	i	i	0.25	32	i	32	256	64	i	i	i	i	i
<i>H. influenzae</i>	16	0.01	128	128	i	i	i	0.01	64	i	32	256	64	i	i	i	i	0.5

Activity is given as minimum inhibitory concentration (MIC) in $\mu\text{g ml}^{-1}$. i = MIC > 256 $\mu\text{g ml}^{-1}$. Activity was determined by an agar incorporation method. Approximately 10^4 cells, surface inoculated onto an amino acid based medium containing doubling dilutions of the compound were incubated for 18 h at 37 °C. The MIC was that which inhibited > 99.9% of the inoculum. Strains used: *Escherichia coli* NCTC 10418, *Klebsiella aerogenes* K5, *Staphylococcus aureus* NCIB 8625, *Streptococcus faecalis* S98, *Haemophilus influenzae* NCTC 4560.

Table 2 *In vitro* antibacterial spectrum of alaphosphin

Organism	ID ₅₀ (broth) Alaphosphin	Alaphosphin	MIC (agar) Pen G	Amp
<i>E. coli</i> E73	0.05	0.06	16	2
<i>E. coli</i> amp E30	0.10	0.06	i	i
<i>K. aerogenes</i> K5	0.5	0.5	32	32
<i>Str. faecalis</i> S98	1.0	1	0.25	1
<i>Micrococcus</i> NCTC 7526	1.0	2	< 0.12	< 0.12
<i>Enterobacter</i> E98	1.3	4	i	i
<i>Serratia marcescens</i> S42	1.4	4	64	8
<i>S. albus</i> S228	1.6	4	< 0.12	< 0.12
<i>Salmonella typhimurium</i> S394	1.7	8	4	0.5
<i>Citrobacter</i> C4	2.4	2	16	4
<i>S. aureus</i> Schoch S13	3.4	8	< 0.12	< 0.12
<i>Providencia</i> P131	2.6	4	16	32
<i>Shigella flexneri</i> S466	ND	0.25	8	1
<i>H. influenzae</i> H34A	ND	16	0.5	0.4
<i>Neisseria gonorrhoeae</i> N3	ND	16	< 0.12	< 0.12
<i>Bacillus subtilis</i> B8	140	16	< 0.12	< 0.12
<i>Proteus mirabilis</i> P92	11	i	4	2
<i>Pseudomonas aeruginosa</i> NCIB 8295	26	i	i	i
<i>Str. pyogenes</i>	ND	i	< 0.12	< 0.12

i = MIC > 256 µg ml⁻¹. ND, not determined. Pen G, penicillin G. Amp, ampicillin. Agar dilution MICs were determined as in Table 1. ID₅₀ was determined by the broth dilution method. Approximately 10⁶ cells were inoculated into a defined amino acid liquid medium (5 ml) containing doubling dilutions of alaphosphin and incubated for 18 h at 37°C. The ID₅₀ was the concentration which inhibited growth at 50% of the control.

is to be expected that alaphosphin will synergise the action of a penicillin such as ampicillin and of D-cycloserine. We have confirmed this *in vivo* and *in vitro* for various bacterial species, including *Klebsiella aerogenes*, *Enterobacter* sp., *Serratia marcescens*, *Salmonella typhimurium*, and *Staphylococcus aureus*.

J. G. ALLEN
F. R. ATHERTON
M. J. HALL
C. H. HASSALL
S. W. HOLMES
R. W. LAMBERT
L. J. NISBET
P. S. RINGROSE

Roche Products Limited,
Welwyn Garden City,
Hertfordshire, UK

Received 12 October 1977; accepted 6 January 1978.

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X-ray dose fractionation and oncogenic transformations in cultured mouse embryo cells

THE effect of dose fractionation on oncogenic transformation can be studied quantitatively by using recently developed cell lines in which transformation is scored by the loss of contact inhibition^{1,2}. We report here that splitting the dose into two fractions enhances oncogenic transformation at low doses, whereas at high doses fractionation results in less effect than the same total dose in a single exposure. This observation

is pertinent to the calculation of cancer risk estimates for multiple low-dose exposures, to which radiation workers or members of the general public are exposed, by means of a linear extrapolation from observed human data at high dose levels.

The cells used were C3H 10T½ cl 8 mouse embryo cell line developed by Reznikoff *et al.*^{1,2}. Cell cultures were maintained in Eagles' basal medium, supplemented with 10% heat-inactivated foetal calf serum, and with penicillin (50 units ml⁻¹) and streptomycin (50 µg ml⁻¹) to minimise contamination. Cells were plated 18 h before irradiation at a density such that approximately 400 viable cells per 100-mm Petri dish survived the various experimental treatments. Plating efficiencies varied from 8% to 16%. Irradiations were performed at room temperature using a Siemens Stabilipan X-ray machine, operating at 300 kVp, 12 mA and having added filtration of 0.2 mm Cu. The dose rate to the cells was in the range 88 to 102 rad min⁻¹. Morphologic transformation was scored as described by Reznikoff *et al.*^{1,2}. Type I foci, seen as dense groupings of cells, were not scored as transformants. Types II and III, dense piling of cells with criss-crossing of polar fibroblastic appearing cells, were scored as transformants (Fig. 1). No spontaneous transformations were observed in the untreated control dishes. The morphological appearance of a transformed clone was consistent with those of Terzaghi and Little¹⁰.

The experimental plan was to compare a single dose with the same dose divided into two equal fractions (doses separated by 5 h), within a large self-contained experiment. This was then repeated at various dose levels from 30 to 800 rad. Because of the large number of Petri dishes needed to compare single and split exposures at one dose level (typically 150 dishes per point) it was not practical to investigate all dose levels in a single experiment. The data obtained in this series of experiments are summarised in Table 1 and plotted in Fig. 2. Types II and III transformed clones were scored separately and are listed in Table 1. There is no obvious difference in the ratio of the two types of transformants produced by single versus split doses, nor does the ratio vary with dose level. Consequently, the conclusions reached from these results would be unaffected if Type III transformants only were scored. The interesting and important result is that the dose-response curves for the induction of transformations by single and split doses cross at about 150 rad. Below this dose level, split doses produce more transformations than a single exposure, while

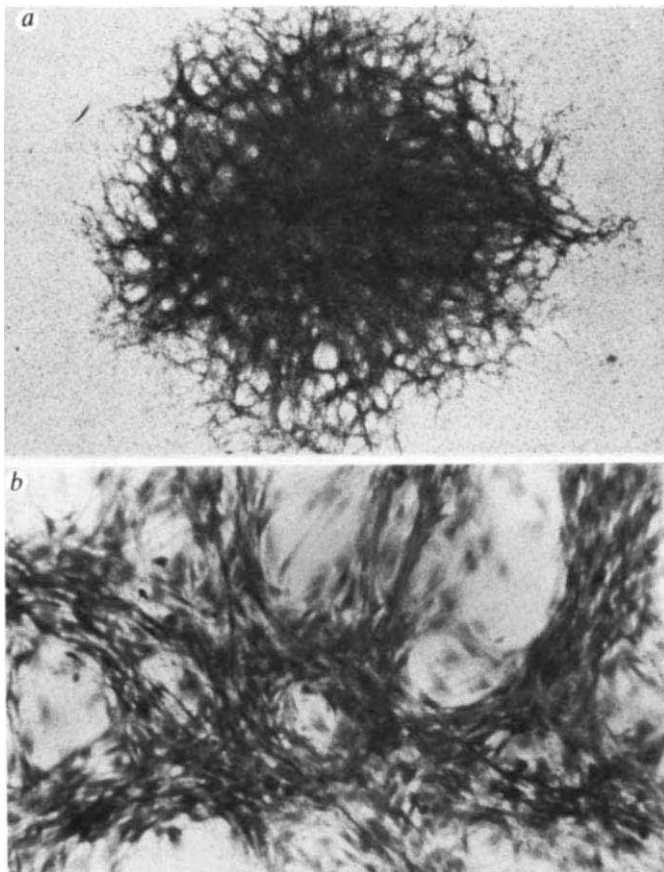


Fig. 1 *a*, Photograph of a type III transformed focus showing dense piling of cells with criss-crossing of polar fibroblastic appearing cells at the edges of the colony ($\times 15$). *b*, View of the edge of a transformed colony at higher magnification showing more clearly the criss-cross pattern ($\times 120$).

above this dose level, split doses are less effective than a single exposure.

Interestingly, the transformation rate for split doses can be calculated from that for single doses by a simple rule. The

incidence for a fractionated treatment (total dose D) is double that for a single exposure of dose $D/2$. This implies that the two dose fractions, separated by 5 h, are independent and that there is no interaction between them.

The transformation data reported here, corresponding to doses above 150 rad, are in remarkable agreement with those of Terzaghi and Little³, who also used C3H 10T $\frac{1}{2}$ cells for split dose experiments. They reported that for total doses in the range 300 to 800 rad, splitting the dose into two equal fractions separated by 5 h, resulted in fewer transformed colonies than a single exposure. Further, a dose of 150 rad seemed to induce the same frequency of transformation whether delivered as a single exposure, or in two equal fractions separated by 5 h. For the lower dose range, however, the data are consistent with those of Borek and Hall⁴ who studied the effect of split doses of X rays on the induction of neoplastic transformations in fresh explants of golden hamster embryos. They found that two doses of 25 rad, separated by an interval of 5 h, produced a higher frequency of transformations than a single dose of 50 rad; likewise, two doses of 37.5 rad separated by the same time interval was more effective than a single dose of 75 rad in inducing neoplastic transformations. The apparent conflict between the enhancement of transformation by dose fractionation reported by Borek and Hall⁴, and the sparing effect of fractionation reported by Terzaghi and Little³ is resolved by our study which shows that the dose-response curves for the induction of transformations by single and split doses cross at about 150 rad.

These results have an important bearing on the practical problem of radiation protection. When either radiation workers or members of the general public are exposed to ionising radiation, it is usually in the form of repeated small doses over an extended period, rather than a single acute exposure to a large dose, as commonly used in experimental studies of carcinogenesis in laboratory animals. For most radiobiological endpoints, including cell killing, dose fractionation and protraction lead to a substantial reduction in the consequences of a given X-ray dose^{5,6}. The opposite seems to be the case for low doses of X rays when transformation is the endpoint observed. This demonstration of an increased incidence of transformation when low X-ray doses are divided must be a factor to be considered in setting radiation protection levels,

Table 1 Oncogenic transformation in C3H 10T $\frac{1}{2}$ cl 8 cells given various X-ray doses

Treatment (rad)	S/S_0	No. of transformation		Total no. of transformed foci/ no. of surviving cells	Average frequency of transformation
		Type II	Type III		
30	0.91	0	5	$5/4.04 \times 10^4$	1.24×10^{-4}
15+15	0.91	2	13	$15/6.0 \times 10^4$	2.50×10^{-4}
50	0.91	0	4	$4/2.92 \times 10^4$	$1.33 \times 10^{-4} \pm 0.04 \times 10^{-4}$
	0.98	2	3	$5/3.86 \times 10^4$	
25+25	0.90	8	6	$14/4.93 \times 10^4$	$2.46 \times 10^{-4} \pm 0.39 \times 10^{-4}$
	0.98	4	8	$12/5.79 \times 10^4$	
100	0.92	4	6	$10/6.17 \times 10^4$	$1.80 \times 10^{-4} \pm 0.18 \times 10^{-4}$
	0.93	3	5	$8/4.05 \times 10^4$	
50+50	0.97	4	9	$13/4.83 \times 10^4$	
	0.91	4	4	$8/2.51 \times 10^4$	$2.94 \times 10^{-4} \pm 0.25 \times 10^{-4}$
200	0.69	0	7	$7/1.87 \times 10^4$	$2.99 \times 10^{-4} \pm 0.76 \times 10^{-4}$
	0.66	1	2	$3/1.14 \times 10^4$	
100+100	0.87	0	4	$4/1.08 \times 10^4$	$2.87 \times 10^{-4} \pm 0.83 \times 10^{-4}$
	0.67	2	0	$2/9.60 \times 10^3$	
300	0.47	3	3	$6/9.81 \times 10^3$	6.12×10^{-4}
150+150	0.76	5	9	$14/5.20 \times 10^4$	2.69×10^{-4}
400	0.31	6	2	$8/5.17 \times 10^3$	$1.16 \times 10^{-3} \pm 0.20 \times 10^{-3}$
	0.30	13	21	$34/3.28 \times 10^4$	
	0.43	5	8	$13/1.47 \times 10^4$	
200+200	0.40	0	3	$3/2.40 \times 10^3$	$8.01 \times 10^{-4} \pm 1.00 \times 10^{-4}$
	0.39	8	8	$16/2.20 \times 10^4$	
	0.37	4	5	$9/1.33 \times 10^4$	
800	0.05	4	5	$9/2.50 \times 10^3$	$3.37 \times 10^{-3} \pm 0.24 \times 10^{-3}$
	0.05	7	23	$30/9.59 \times 10^3$	
400+400	0.13	0	3	$3/3.70 \times 10^3$	$1.19 \times 10^{-3} \pm 0.37 \times 10^{-3}$
	0.16	5	12	$17/1.09 \times 10^4$	

S/S_0 , no. of cells after X-ray exposure/no. of cells at zero time.

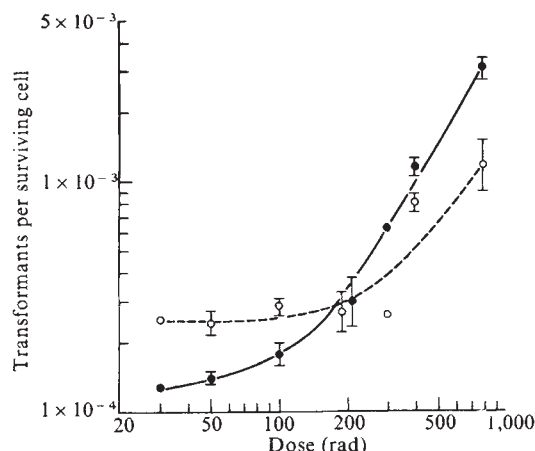


Fig. 2 Dose-response relationships for the number of transformants per surviving cell following single (●) or split doses (○) of X rays. In the case of split doses, the radiation was delivered in two equal exposures separated by 5 h.

or in assessing the risk to the public from multiple low-dose radiation exposures such as those involved in the medical uses of X rays.

This study involves, of course, an *in vitro* model system. Comparison of *in vitro* results with similar studies of tumour induction in laboratory animals is interesting, but fraught with difficulties. Most studies of tumour induction in animals have involved larger doses and longer time intervals than those used in this study. Kaplan and Brown^{7,8} found that 475 rad, given to mice as four exposures at 4- or 8-d intervals, was more tumorigenic than the same dose given as a single exposure, or in daily fractions. In contrast, Metalli *et al.*⁹ concluded that split doses of sublethal irradiation with short fractionation intervals are less efficient inducers of tumours and leukaemias than a single dose. Upton *et al.*¹⁰ found a complex pattern of X-ray induced myeloid leukaemia in mice that was intermediate between these two sets of results. The advantage of the *in vitro* study, compared with most animal experiments, is that in general, a larger dose range can be investigated, which can in particular be extended to quite low doses.

If the observation *in vitro* that splitting the dose enhances transformation at low doses, and spares at high doses, can be assumed to apply *in vivo*, in the human, it has important practical implications. In particular, it is pertinent to the calculation of cancer risk estimates for multiple low-dose exposures, by means of a linear extrapolation from observed human data for single high doses. If fractionation enhances transformation at low dose levels, the linear extrapolation does not necessarily constitute a conservative estimate, or upper limit, as assumed in the BEIR report¹¹.

We thank Dr Harald H. Rossi for useful discussions. This work was supported by contract EY-76-02-3243 from the US Department of Energy and by grants CA-09053 and CA 12536 from the NCI, DHEW.

RICHARD MILLER
ERIC J. HALL

Radiological Research Laboratory,
College of Physicians and Surgeons,
Columbia University,
New York, New York 10032

Received 3 November 1977; accepted 3 January 1978.

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Enhanced survival of ultraviolet-irradiated herpes simplex virus in carcinogen-pretreated cells

WHEN CV-1 monkey kidney host cells are exposed to low doses of ultraviolet (UV) or X-ray radiation, or to photodynamic treatment before infection with herpes simplex virus type 1 (HSV), the survival of UV-irradiated virus is increased¹⁻³. This increase is more pronounced if the time interval between cell UV-irradiation and virus infection is increased by a few days⁴. A similarly increased survival of UV-damaged phage λ is observed in bacteria damaged by UV, X rays, thymine starvation, exposure to mitomycin C, or thermal shift of *tif-1* mutants⁵. Moreover, it has been shown that enhanced survival of UV-damaged phage λ occurred in bacteria previously treated with enzymatically activated aflatoxin B₁, a potent hepatocarcinogen⁶. The purpose of this study was to determine whether treatment of CV-1 cells with carcinogens would result in enhanced survival of UV-inactivated HSV. We chose for this purpose (1) aflatoxin B₁ because, after treatment with this activated mycotoxin, human fibroblast cells exhibit a UV-like repair replication, the extent of which is at the very most 20% of that observed after UV irradiation⁷, and (2) acetylaminofluorene (AAF) and its derivatives, *n*-acetoxy-2-AAF and *n*-hydroxy-2-AAF, as these derivatives are known to be active intermediates in the carcinogenic activity of the parental compound⁸, and because, in *n*-acetoxy-AAF-treated human cells, there is a UV-like repair response⁹. Virus reactivation was

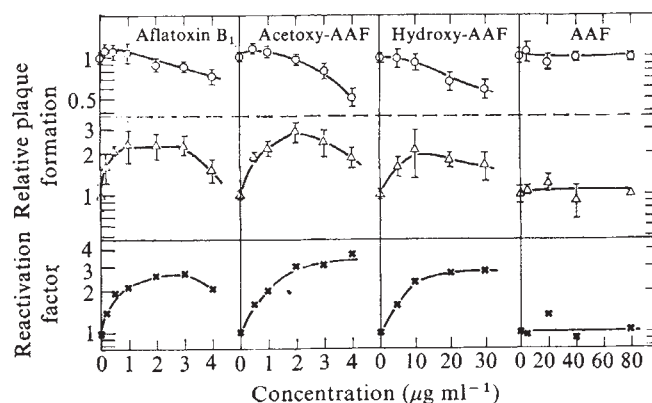


Fig. 1 Effect of different concentrations of aflatoxin B₁ (Sigma), *n*-acetoxy-AAF, *n*-hydroxy-AAF, and AAF on plaque formation by UV (450 J m⁻²)-irradiated (Δ) and control (○) herpes simplex virus in CV-1 monkey kidney cells. Cell monolayers in T-30 plastic tissue culture flasks (Falcon) were rinsed with Dulbecco's PBS, incubated at 37 °C for 30 min with 2 ml of the appropriate concentration of the carcinogen in Dulbecco's PBS, rinsed, and then the reactivation activity present in the cells assayed by immediate virus infection. Culture medium, cell culture and virus assay techniques and irradiation procedures have been described elsewhere⁴. Results of typical experiments are presented; the errors bars denote standard deviations. The curves for un-irradiated virus represent the capacities of treated cells to support virus plaque formation. The curves for irradiated virus demonstrate enhanced survival in treated cells, except for AAF treatment. The reactivation factor (ratio of the above values for irradiated virus to those for control virus) (×) is a quantitative measure of the increased virus survival. The surviving fraction of irradiated virus in untreated cells was 0.0015.

assayed in the same manner as that used in previous investigations of radiation-enhanced reactivation¹⁻⁴.

Freshly confluent monolayers of CV-1 TC7 monkey kidney cells were treated with appropriate concentrations of the compounds, and the treated cells were then infected with UV-irradiated or control (unirradiated) HSV (MP strain) at various times after treatment⁴. Enhanced reactivation was quantified by calculating the reactivation factor (RF), that is, the ratio of the surviving fraction of the virus in treated cells to that in untreated cells⁴. The RF depends on the UV dose to the virus¹⁰, and so the RF is a relative, not an absolute, value. Therefore, a single UV dose to the virus was used throughout this study.

Figure 1 shows the effects on plaque formation of treatment of host cells with different concentrations of carcinogens. Plaque formation by UV-irradiated virus increased with cell treatment, resulting in increases of RF to two to four for aflatoxin B₁, acetoxy-AAF and hydroxy-AAF, whereas the RF remained at one in cells treated with AAF. Thus, treatment of the cells with three of four carcinogens enhances survival of UV-irradiated herpes virus.

The curves (Fig. 1) for control virus represent the capacity of the treated cells to support herpes virus plaque formation. The inactivation of the cell capacity reflects the toxicity of the chemical treatment. Thus, in these experimental conditions, the carcinogens were found to be toxic at concentrations above 1 µg ml⁻¹ for aflatoxin B₁, 2 µg ml⁻¹ for acetoxy-AAF, and 5 µg ml⁻¹ for hydroxy-AAF. AAF, however, was not toxic at any concentration up to 80 µg ml⁻¹, which exceeded its solubility limit. Moreover, exogenous enzymatic activation of aflatoxin B₁ was not needed to obtain an increase of the RF (Table 1). Table 1 also shows that cell treatment with 1% dimethylsulphoxide in phosphate-buffered saline (control solution) was without effect on plaque formation by control or UV-irradiated virus.

Previous experiments with UV radiation have shown that the extent of enhanced virus survival is higher with increasing time intervals between cell irradiation and virus infection⁴. A time-dependent increase of enhanced survival was also observed after carcinogen treatment of the cells. Figure 2 shows the RF for virus infection at different times after cell treatment. The concentrations of carcinogens used were chosen as the highest levels which did not affect the capacity of cells to support plaque formation by unirradiated virus (see Fig. 1). A similar low dose of UV radiation was used for comparison. Treatments with higher concentrations of carcinogens or greater doses of

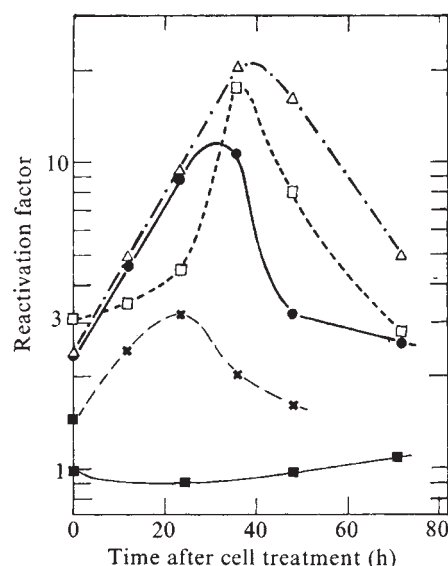


Fig. 2 Reactivation factors at various times after cell treatment by different carcinogens and ultraviolet radiation, as determined by infection and subsequent plaque formation by UV-irradiated (450 J m⁻²) herpes simplex virus in CV-1 monkey kidney cells. Cell treatment procedure and virus assay were as described in Fig. 1 for the following cell treatments: aflatoxin B₁, 1 µg ml⁻¹ (●); *n*-acetoxy-AAF, 1 µg ml⁻¹ (△); *n*-hydroxy-AAF, 7 µg ml⁻¹ (□); AAF, 40 µg ml⁻¹ (■); ultraviolet radiation, 4 J m⁻² (×). The data presented are averages from two to four experiments; error bars were deleted for clarity of presentation.

UV were not used because the cell monolayer regrowth which occurs after cell killing may be accompanied by higher virus survival¹¹. We wished to avoid this complication because we are primarily interested in potentially inducible enhanced survival.

In all cell treatments, the RF increased with delayed infection for 24–36 h, then decreased. The time courses of the reactivation observed after carcinogen treatments were similar to that occurring after UV irradiation of the CV-1 cells (Fig. 2), as was also found with treated *Escherichia coli* infected with UV-irradiated phage λ¹², suggesting that the enhanced reactivations could depend on a common process. The enhanced survival was much more pronounced in carcinogen-treated cells than in UV-irradiated ones, perhaps because the chemical damage is not as efficiently removed by excision–repair processes^{7,13}.

Aflatoxin B₁ is stable in water, and so it was decided to expose cultures to very low concentrations of aflatoxin B₁ for 24 h to determine the lowest concentration of this compound giving an effect in these conditions. The data shown in Fig. 3 demonstrate that this enhanced survival system (HSV and monkey kidney cells) can be used to detect aflatoxin B₁ concentrations at least as low as 1 ng ml⁻¹.

Our results show enhanced survival of UV-irradiated herpes virus in CV-1 cells previously treated with low concentrations of aflatoxin B₁ or carcinogenic derivatives of AAF, *n*-acetoxy-2-AAF and *n*-hydroxy-2-AAF. Sarasin and Hanawalt¹⁴ have obtained enhanced survival of UV-irradiated SV40 virus in CV-1 cells treated with four carcinogens, including aflatoxin B₁ and acetoxy-AAF. Hellman (personal communication) has preliminary evidence that 4-nitroquinoline-1-oxide can also enhance survival of UV-irradiated herpes virus in CV-1 cells.

Our results show that CV-1 cells can metabolise aflatoxin B₁ into an active compound, as already observed for rodent kidney cells treated *in vivo*¹⁵. On the other hand, our data suggest that CV-1 cells did not metabolise AAF into a compound as active as either of the AAF derivatives tested here. This is consistent with studies using hamster kidney cells which indicate that acetoxy-AAF and hydroxy-AAF are more active *in vitro* than AAF^{16,17}. Perhaps CV-1 monkey kidney cells can metabolise aflatoxin but not AAF.

Table 1 Lack of effect of DMSO* or of NADPH/liver microsome activation mixture† on aflatoxin-enhanced survival of UV-irradiated herpes virus

Cell treatment‡	Relative plaque formation		Reactivation factor
	Unirradiated virus	UV-irradiated virus§	
Control (Dulbecco's phosphate-buffered-saline)	1.00±0.05	1.00±0.09	1.00±0.14
1% DMSO	1.06±0.07	1.12±0.05	1.06±0.12
Activation mixture	1.13±0.01	1.47±0.28	1.30±0.29
Aflatoxin B ₁ (1 µg ml ⁻¹ in 1% DMSO)	0.96±0.08	2.10±0.25	2.19±0.33
Aflatoxin B ₁ (1 µg ml ⁻¹ in 1% DMSO)+activation mixture	1.10±0.05	1.71±0.25	1.55±0.30

* Dimethylsulphoxide in Dulbecco's phosphate-buffered saline.

† 16.5 ml of NADPH activation mixture (12 mM MgCl₂, 6 mM NADP, 8 mM glucose-6-phosphate, 1 unit ml⁻¹ glucose-6-phosphate dehydrogenase in Dulbecco's PBS) with 3.3 ml of post-mitochondrial fraction (S9) of livers from Aroclor 1254 treated Sprague-Dawley rats (Litton Bionetics).

‡ Cells were treated with 2 ml for 30 min at 37 °C.

§ 450 J m⁻²; surviving fraction in control cells was 0.0015.

|| Uncertainty expressed as standard error.

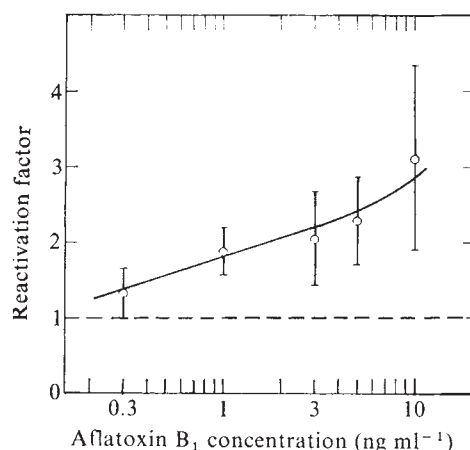


Fig. 3 Reactivation factors for UV-irradiated (450 J m^{-2}) herpes simplex virus infecting CV-1 monkey kidney cells after 24 h incubation at 37°C with 4 ml of tissue culture medium containing different concentrations of aflatoxin B₁. The data represented are the means and standard deviations of values from four separate experiments.

The enhanced survivals caused by the carcinogens were similar in both magnitude and time course. The primary difference was in the concentration of compound needed to elicit the response, with the most carcinogenic, aflatoxin B₁, being effective at the lowest concentrations. There seems to be a striking parallelism between these results and between those with oncogenic cell transformation and with prophage induction. Pienta *et al.*¹⁶ and Ishii *et al.*¹⁷ have shown that active intermediates of carcinogens can most effectively oncogenically transform hamster kidney cells. Moreau *et al.*¹⁸ found a good correlation between the prophage inducing activity and the carcinogenic activity of several carcinogenic compounds. The enhanced reactivation system of herpes simplex virus and CV-1 monkey kidney cells could be used as a screening system for testing carcinogenic potential of chemical compounds in mammalian cells. The sensitivity (1 ng of aflatoxin B₁) and the short assay time (3 d) of this particular system are distinct advantages over other mammalian systems.

An hypothesis to explain the mechanism of carcinogenesis has been proposed by Radman¹⁹ and Devoret²⁰, using data derived from bacterial studies on induced 'SOS' functions (see review by Witkin²¹). A major element of this hypothesis is the induction of 'repair' which is error-prone. It was postulated²² that in eukaryotes, carcinogens may induce cellular SOS functions similar to those in *Escherichia coli*. In bacteria, one such SOS function is induced phage reactivation²¹. Enhanced reactivation of herpes virus in mammalian cells may be analogous to induced phage reactivation. The data presented here, demonstrating that carcinogens give rise to enhanced reactivation, support the above postulate.

We thank R. Devoret for suggesting this study, A. Sarasin for discussing his unpublished data, L. E. Bockstahler for his encouragement, and J. G. Goddard for technical assistance. Acetoxy-AAF and hydroxy-AAF were from J. A. and E. C. Miller of McArdle Lab., University of Wisconsin. W.D.T. was supported by NCI grant CA 18970.

C. DAVID LYTLE

Bureau of Radiological Health,
Food and Drug Administration,
Department of Health, Education, and Welfare,
5600 Fishers Lane,
Rockville, Maryland 20857

JACQUES COPPEY

Foundation Curie-Institut du Radium,
Section de Biologie,
26, Rue d'Ulm,
75231 Paris Cedex 05, France

WILLIAM D. TAYLOR

Department of Biochemistry and Biophysics,
Pennsylvania State University,
University Park, Pennsylvania 16802

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Human spontaneous killer cells selective for tumour-derived target cells

DURING the last few years, it has become clear that normal individuals have a lymphocyte subpopulation which has the capacity to kill certain *in vitro* propagated cell lines¹⁻³. The effector cells involved in this kind of cytotoxicity have been designated either natural or spontaneous (SK) killer cells and have mainly been defined using lymphocytes derived from the peripheral blood. It has been shown that the majority of SK cells have receptors for the Fc part of IgG, and that some have receptors for the activated third complement factor (C3), but little is known about the specificity, recognition and effector mechanisms underlying spontaneous cytotoxicity¹⁻³⁻⁴. Although the actual biological significance of SK cell cytotoxicity is unclear, it has been proposed that these killer cells act *in vivo* as surveillance cells against tumour development. We give here evidence in support of this idea, for we have found that SK cells, in a short-term cytotoxicity assay, preferentially kill cell lines derived from leukaemic tumours as compared to cell lines from normal lymphocytes.

All cell lines investigated were of the suspension type, cultured in RPMI-1640 with 10% FCS and antibiotics, and subcultured twice weekly. The origin and classification of the various lines are given in Table 1. All the tumour-derived B cell lines originated from Burkitt's lymphoma and carried the Epstein-Barr virus (EBV) genome except for the BJAB line, which originates from a rare case of EBV-negative African Burkitt's lymphoma. The T cell lines are both from acute lymphatic leukaemia. The K-562 line is derived from chronic myeloid leukaemia and is the only line so far established from this disease. All cell lines derived from normal lymphocytes were established either spontaneously or by the addition of transforming EBV. The cytotoxicity assay used was the short-term (3-5 h) ⁵¹Cr release assay performed in conical microplates (see text to Fig. 1). Effector lymphocytes were isolated from whole blood by buoyant density centrifugation on Ficoll-Isopaque, and phagocytic cells removed by the iron carbonyl powder technique⁵. Lectin-induced cytotoxicity was determined by the addition of concanavalin A (Con A) at a final concentration of $1 \mu\text{g ml}^{-1}$. The spontaneous ⁵¹Cr release in the absence of

Table 1 Spontaneous cytotoxicity and Con A-induced cytotoxicity against cell lines derived from normal lymphocytes or from leukaemic tumour cells

Target cell	Origin	Characteristics	Presence of EBV genome	Spontaneous cytotoxicity (mean %) [†] Range	Specific Con A-induced cytotoxicity (mean %) [‡] Range
P3HR-I	Burkitt's lymphoma ¹⁴	B cell	Yes	33 (9) 15-62	24 (7) 13-42
BJAB	Burkitt's lymphoma ¹⁵	B cell	No	36 (4) 21-58	26 (4) 18-31
Daudi	Burkitt's lymphoma ¹⁴	B cell	Yes	34 (5) 16-71	ND
Raji	Burkitt's lymphoma ¹⁴	B cell	Yes	4 (7) (-5)-8	36 (7) 26-63
Molt-4	Acute lymphocytic leukaemia ¹⁶	T cell	No	61 (10) 28-76	16 (8) 5-28
HSB-II	Acute lymphocytic leukaemia ¹⁷	T cell	No	42 (3) 18-67	11 (3) 5-17
K-562	Chronic myeloid leukaemia ¹⁸	CML cell	No	59 (13) 19-70	ND
322	Normal lymphocytes§	B cell	Yes	2 (3) 1-4	26 (3) 20-31
323	§	B cell	Yes	6 (3) 0-7	43 (3) 33-49
325	§	B cell	Yes	1 (3) (-2)-2	24 (3) 15-29
327	§	B cell	Yes	3 (3) 2-5	31 (3) 26-41
LA-85	§	B cell	Yes	10 (3) 6-12	32 (1) 32
LA-109*	§	B cell	Yes	10 (4) 8-11	44 (2) 36-52
LA-237	§	B cell	Yes	7 (3) 3-10	48 (1) 48
LA-253	§	B cell	Yes	3 (7) 0-5	16 (16) 7-21
LCL-1		B cell	Yes	-7 (2) (-9)-(-5)	ND
LCL-2B		B cell	Yes	-6 (2) (-7)-(-5)	ND
LCL-7		B cell	Yes	3 (2) 2-4	ND
LCL-12		B cell	Yes	0 (2) (-1)-1	ND
LCL-14		B cell	Yes	5 (2) 3-7	ND
Wil-2	See ref. 7	B cell	Yes	4 (4) 2-6	33 (4) 21-41
NC-37	See ref. 19	B cell	Yes	9 (10) 4-11	ND

*LA-109 was established from a leukaemic patient, but probably originates from normal B lymphocytes.

[†]Numbers within parentheses indicate numbers of tested lymphocyte donors. Cytotoxicity was determined by a 3-5 h ⁵¹Cr release assay as described in text to Fig. 1 using a lymphocyte : target ratio of 20 : 1.

[‡]Specific Con A-induced cytotoxicity is given, thus the % of spontaneous cytotoxicity is subtracted from each value.

§These cell lines were established at the Department of Microbiology and Immunology, UCLA, by Drs C. Spina and M. Jobin.

||These cell lines were established by Dr Richard A. Gatti at the Department of Pediatric Oncology, Cedars-Sinai Medical Center, Los Angeles.

effector lymphocytes ranged over 1-5% h⁻¹. Experiments with a higher spontaneous release than 25% were not included in the present investigation.

Two representative experiments are shown in Fig. 1. Here it is clear that the spontaneous cytotoxicity is many times stronger against the tumour-derived cell lines P3HR-I, Molt-4, HSB-II, and K-562 than against the cell lines derived from normal lymphocytes 109, 237 and Wil-2. In the tumour-derived group, the Raji line is an exception, as it is virtually resistant to SK cell cytotoxicity. Table 1 summarises several experiments done with different donors on different occasions, and further confirms the difference in susceptibility to spontaneous cytotoxicity between the tumour-derived cell lines and the lines derived from normal lymphocytes. The lack of susceptibility on the part of the normal cell lines is not correlated with time in culture, as the two long-term cultured lines Wil-2 and NC-37 are killed in a range similar to that of the other lines cultured for a shorter time period (less than two years), and there is no gross difference in specific Con A-induced cytotoxicity between the two groups, demonstrating that the cell lines in the normal group are not *per se* resistant to cell-mediated cytotoxicity. The cell line 253 may be an exception, as there is little induction of lectin-induced cytotoxicity against this particular line. The relatively low Con A-induced cytotoxicity against the cell lines that are highly susceptible to SK cytotoxicity (Mol-4 and HSB-II) is related to the fact that these cell lines are killed so efficiently by the SK cells that additional lectin-dependent killing is hidden. In a separate series of experiments, we have found a higher degree of Con A-induced cytotoxicity with lymphocyte populations depleted of SK cells (by removal of Fc receptor-bearing lymphocytes) against Molt-4 and HSB-II.

From our experiments, it seems that cell lines derived from leukaemic tumour cells are much more susceptible to spontaneous cytotoxicity than cell lines derived from normal lymphocytes. This difference to susceptibility is most convincingly demonstrated with regard to leukaemic cells of B cell origin, as the normal cell lines are all of the B cell type and all carry EBV. For the T cell lines (Molt-4 and HSB-II) and for the CML line (K-562), there are no corresponding normal cell lines, although it has been reported that normal T cells can be tissue cultured

for long periods if regularly fed with growth-promoting factors derived from supernatants of PHA-stimulated lymphocytes⁶. It will thus be of great interest to investigate if these long-term cultured normal T cells show an increased susceptibility to SK cytotoxicity, or if the distinction between normal and malignant cells also holds true for the T cell-derived tumours. The latter is most likely, however, as we have so far detected very little or no cytotoxicity against mitogen-induced T lymphoblasts (data not shown).

With regard to cells of B cell origin, there is much evidence that the cell lines derived from normal cells retain a basically normal character *in vitro*, while the leukaemic cell lines do not: leukaemic B cells are monoclonal in contrast to normal cell lines, which initially are polyclonal^{7,8}; the morphology and growth pattern of malignant cells are distinguishable from those of normal cells⁹; lines derived from normal cells have a normal karyotype, whereas tumour-derived B lines often have chromosomal aberrations (a chromosome 14-translocation)^{9,10}; tumour-derived cell lines often grow in immunoincompetent, T cell-deficient nude mice, in contrast to normal cell lines¹¹; it has been shown that a high number of cells from a normal cell line can be injected into the same person from which the line initially was established without causing a leukaemic state^{12,13}. These experiments were done on terminal cancer patients, and further argue for the relative harmlessness and nonmalignant character of these cells.

The seemingly normal phenotype of the common EBV-dependent cell lines may offer an interesting possibility to activate SK cells *in vivo*. We have recent evidence which demonstrates that autologous B cell lines of normal origin have the capacity to stimulate peripheral lymphocytes, in MLC-type cultures *in vivo*, to increased DNA synthesis and generation of killer cells with the same specificity as that described for SK cells in this report. Although we feel that this is an important finding, which may offer a way of activating SK cells *in vivo*, it is clear that far too little is known about the SK system at the present to consider any clinical trials in this direction.

The mechanism behind SK cytotoxicity remains unclear; consequently, it is also unclear why SK cells kill tumour-derived target cells more efficiently than normal targets. Several explana-

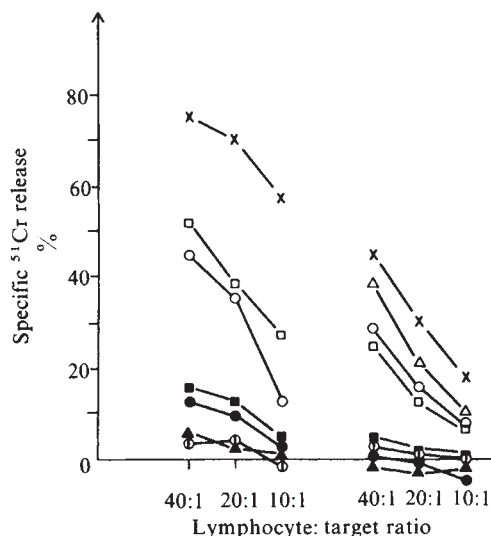


Fig. 1 Spontaneous cytotoxicity against cell lines of normal or leukaemic origin, using effector lymphocytes from two individuals. Cytotoxicity was determined by ^{51}Cr release. Labelled target cells (10^4) were mixed with freshly isolated allogeneic lymphocytes at different lymphocyte : target ratios, in a total volume of 150 μl of RPMI medium with 10% FCS in standard conical 96 hole microplates (Cooke Laboratory Products, Cat. No. 1-220-25A). The plates were then incubated at tissue culture conditions for 3–5 h, whereafter 50 μl of supernatant was collected from each well and cytotoxicity determined by the amount of released radioactivity, according to the standard formula. The target cells were K-562 (Δ), Molt-4 (X), HSB-II ($\circ$), P3HR-1 ($\square$), Raji ($\blacktriangle$), 109 ($\blacksquare$), 237 ($\bullet$), and Wil-2 (Φ). The origin and characteristics of the target cells are given in Table 1.

tions may be considered: different clones of different sizes of effector cells, a qualitative or a quantitative difference with regard to surface antigen expression on the target cells, or a difference in general surface characteristics, such as charge, mobility and adhesiveness. But whatever the mechanism, the difference in susceptibility between normal B cell lines and cell lines of leukaemic origin seems to be a fact. We feel that this finding may be important for the understanding of the interaction between lymphocytes and malignant or pre-malignant cells, and we are presently trying to define further the specificity with regard to non-leukaemic malignancies, as well as to define *in vitro* conditions for the activation of SK cells.

We thank Miss D. Brown for technical assistance. M.J. received support from NIH grant CA 12800, the Swedish Cancer Society and Cancer Research Institute, Inc. C.S. received NIH grant F32-CA05295 and S.T. USPHS grant T32-A1 07126.

MIKAEL JONDAL
CELSA SPINA
STEPHAN TARGAN

Department of Microbiology and Immunology,
UCLA School of Medicine,
Los Angeles, California 90024

Received 25 October 1977; accepted 9 January 1978.

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Two distinct mechanisms of immune suppression by antibody

THE humoral immune response is controlled by a complex regulatory system. Antigen-reactive B lymphocytes receive essential stimulatory signals from helper T cells (or macrophages) and inhibitory signals from another class of T cells, suppressor T cells. The end product, antibody, also has a regulatory role in that it inhibits antibody production. The latter is not unexpected because antibody represents a solubilised copy of the B lymphocyte's recognition site for antigen, and could thus interfere with the recognition of antigen. The theory that antibody inhibits antibody production by masking antigen¹ cannot explain, however, the observation first made by Sinclair *et al.*^{2,3} that an antigen-unrelated part of 7S antibody, the Fc portion, determines its inhibitory activity. We show here that Fc-dependent antibody-mediated immune suppression is reversed by factors that substitute for helper T cells, while Fc independent immune suppression, active only in high concentrations of antibody, is not affected by helper factors. We conclude that Fc-dependent immune suppression by antibody affects the cooperation of T cells and B cells but not recognition of antigen, and we attribute Fc independent immune suppression by high concentrations of antibody to masking antigenic sites.

We have confirmed that antibody to sheep red blood cells (SRBC) loses much of its inhibitory activity after removal of the Fc portion⁵. Further investigation of this phenomenon indicated that inhibitory antigen-antibody complexes did not affect the activity of helper T cells or antigen-reactive B cells. Evidence for the unimpaired ability of T cells to serve as helper cells was developed by taking advantage of the fact that SRBC and burro red blood cells (BRBC) cross-react on the T-cell level, but, not on the B-cell level⁶. SRBC-primed helper T cells stimulate formation of antibody to both SRBC and BRBC. We have shown that antibody to SRBC (which does not cross-react with BRBC) inhibits the production of antibody to SRBC but does not diminish the helper effect of SRBC-primed T cells on BRBC-reactive B cells⁷. Conversely, trinitrophenol (TNP)-reactive B cells (which respond to TNP conjugated with both SRBC and BRBC) fail to produce antibody to TNP-SRBC in the presence of antibody to SRBC, but remain responsive to TNP-BRBC in these conditions⁵.

As these results indicated that neither T cells nor B cells are the immediate target of suppressive antibody, we proposed that antibody affects the process of cooperation between T cells and B cells by attaching, through its Fc fragment, particulate antigen (with many antigenic sites) to the surface of cells which carry Fc receptors⁷. Such attachment would restrict the availability of free antigen for the cooperation of B cells with helper T cells, but would not affect recognition of antigen by antigen-reactive lymphocytes. This view is consistent with our observation that simultaneous administration of SRBC and antibody to SRBC does not inhibit the generation of SRBC-reactive helper T cells⁸ (while inhibiting the generation of anti-SRBC PFC) which implies again that antibody does not simply act by masking antigen. As T cells respond readily despite the presence of administered antibody, we considered the possibility that B cells may also recognise antigen in the presence of antibody but fail to convert to plaque forming cells (PFC) because their proper association with helper T cells is prevented by antibody. We tested this possibility by substituting helper T cells with other helper signals.

A potent substitute for helper T cells was recently discovered in tumour necrosis serum (TNS). (TNS, obtained from BCG-infected mice 2 h after injection of LPS, is so called because it causes acute necrosis and subsequent regression of tumour grafts in syngeneic mice⁹.) We have shown that TNS supports the production of antibody by cultured B6D2F1 spleen cells depleted of T cells by treatment with anti-Thy-1 serum and

complement, and by cultured nu/nu spleen cells¹⁰. TNS was used as helper signal in the experiments described here.

We tested three types of antibody to SRBC in experiments designed to measure inhibition of the anti-SRBC response in Mishell-Dutton cultures of BDF1 spleen cells: (1) 7S rabbit anti-SRBC, (2) 7S rabbit anti-SRBC F(ab)₂, and (3) chicken anti-SRBC whose Fc portion is not recognised by mammalian cells^{5,11}. While all three preparations inhibited the generation of anti-SRBC PFC at high concentration, only intact rabbit antibody was inhibitory also at low concentration. TNS reversed the suppressive effect of low concentrations, but not high concentrations, of rabbit anti-SRBC antibody. The inhibitory effect of high concentrations of rabbit anti-SRBC F(ab)₂ or chicken anti-SRBC was not reversed by TNS (Fig. 1). Similar results (not shown) were obtained with allogeneic effect factor (AEF, obtained from Drs Amerding and Katz) which, like TNS, replaces helper T cells¹². These results support the notion that antibody can inhibit the cooperation of T cells and B cells⁷—the inhibitory effect is reversed by replacing the signal which results from that cooperation.

In addition, our experiments indicate that, for cellular antigen, two mechanisms of antibody-mediated suppression of antibody production exist: one is operative at low antibody concentration, depends on the Fc portion of the antibody, and is reversed by factors that substitute for helper T cell function; the other requires high concentrations of antibody and is not reversed by factors that replace T cells. The latter mechanism may be based on the masking of antigenic determinants which makes them inaccessible to responsive lymphocytes. The former mechanism provides the possibility that one antibody molecule, reacting with one antigenic determinant of a particulate antigen and, attaching its Fc portion to cellular Fc receptors, removes all other determinants on the same carrier from the circulation

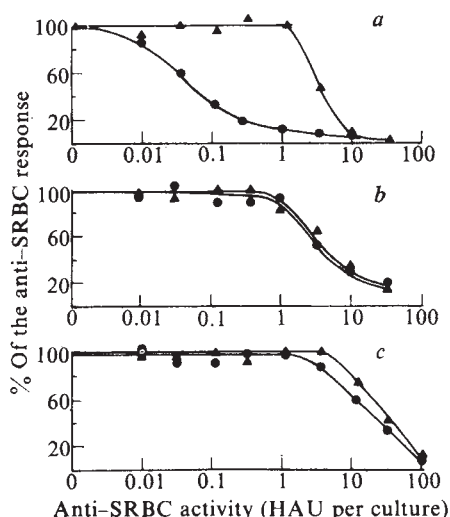


Fig. 1 Effect of T cell-replacing factor in TNS on antibody mediated immune suppression. Spleen cells from (C57BL/6 × DBA/2)F₁ mice were immunised with sheep red blood cells (SRBC) and cultured for 4 d¹⁴. One-half of the cultures received TNS (1%) (▲), the other half did not (●). All cultures (with the exception of control cultures) received anti-SRBC serum in different concentrations. *a*, 7S rabbit anti-SRBC. *b*, 7S rabbit anti-SRBC F(ab)₂ (both antibody preparations obtained from Dr Koo, Memorial Sloan-Kettering Cancer Center). *c*, Chicken anti-SRBC serum, prepared by us with a primary immunisation of one chicken with 2 × 10⁷ SRBC followed by a second immunisation with 2 × 10⁷ SRBC 2 weeks later and exsanguination of the chicken 1 week after the second immunisation. Anti-SRBC antibody activity is expressed in haemagglutinating units (HAU): 1 HAU is defined as the smallest amount of antiserum causing agglutination of 5 × 10⁶ SRBC in a microhaemagglutination assay. Anti-SRBC antibody producing cells were enumerated by a modified¹⁵ Jerne haemolytic plaque assay¹⁰, and the results are given as the percentage of the response in the control culture receiving SRBC. Each point represents a determination made with a pool of three cultures.

Thus attached to cell surfaces, antigen would remain recognisable for T cells and B cells, but could no longer serve as a bridge of communication between them. This concept can apply only to complex antigens with many antigenic determinants and not to soluble antigens. It has in fact been shown that removal of the Fc fragment from anti-BGG antibody does not affect its ability to suppress the production of antibody to BGG⁴. Although these experiments with soluble antigens were performed *in vivo*, they confirm the concept proposed here. Also consistent with this concept is the finding that Fc-dependent antibody mediated immune suppression is not determinant specific⁶, while Fc-independent antibody mediated immune suppression is determinant specific¹³.

This work was supported in part by the US NCI (grants CA 08748 and CA 17673) and by the American Cancer Society (grants IM 82 and IM 87).

MICHAEL K. HOFFMANN

Memorial Sloan Kettering Cancer Center,
1275 York Avenue, New York, New York 10021

JOHN W. KAPPLER

Department of Microbiology,
Division of Immunology,
School of Medicine and Dentistry,
University of Rochester,
Rochester, New York 14642

Received 2 November 1977; accepted 3 January 1978.

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Is serum thymic factor of thymic origin?

THE role of the thymus in the control of the immune response is generally accepted, but the existence and the role of thymic hormones remain controversial¹. Among factors claimed to be thymic hormones^{2–6}, 'facteur thymique serique' (FTS) was not extracted from the thymus gland but from pig's blood⁴. This serum factor is a nonapeptide⁷, and poses the problem of whether FTS, or similar material, is present in the thymus¹. By using non-denaturing techniques (4 °C ultrafiltration and chromatography) for the isolation of peptides and proteins from the thymus we have found that it does not.

The thymus organs were dissected clean immediately after death and homogenised in 0.1 M ammonium bicarbonate, 1 mM 2-mercaptoethanol, pH 8.0, 30% wet weight per volume. Cell debris was removed by centrifuging at 14,000g for 30 min. The supernatant was ultrafiltered twice on an Amicon DH-4 hollow fibre system successively fitted with H1X50 and H1DP10 cartridge to separate the proteins into three fractions with molecular weights (MW) above 50,000, between 10,000 and 50,000, and below 10,000. The three fractions were lyophilised and assayed for their biological activity in the test used to identify FTS (T-rosette)⁸. Calf and pig thymus were studied.

Biological activity was found exclusively in the 0–10,000 MW range ultrafiltrates, using a sensitive (1 ng ml⁻¹) nude mice T-rosette test. False positive results due to the high nucleic acid content of the thymus were excluded by running the T-rosette test after the addition of DNase and RNase to the

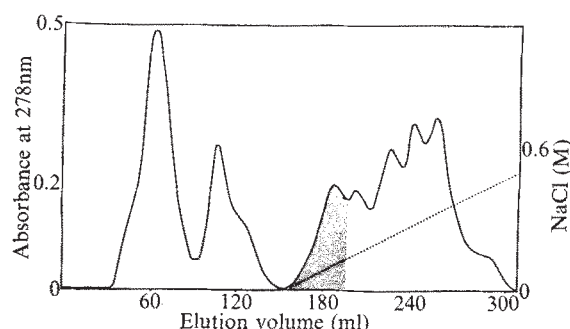


Fig. 1 Diethylaminoethyl Sephadex A-25 chromatography of calf thymus fractions. The 1.5×20 cm column was equilibrated with 50 mM Tris-HCl, 1 mM 2-mercaptoethanol, pH 8.0. Desalted and lyophilised active fractions from the carboxymethyl Sephadex step were dissolved in 5 ml equilibration buffer and loaded on top of the column, and elution was started with equilibration buffer. When all unbound material was excluded from the gel, a linear gradient of 150 ml zero M–150 ml 1.0 M NaCl in Tris buffer was developed. Active material (shaded areas) was eluted with 0.19–0.21 M NaCl (titration of chloride ion). Temperature 4°C , flow rate 40 ml h^{-1} . Outflow was monitored with an LKB Uvicord III photometer. The same conditions were used for the pig thymus fractions, and similar elution profiles were obtained, active material being excluded with 0.15 M NaCl.

extracts⁸. In addition, the inactivation of the extracts by Pronase or papain suggests the peptide nature of the active material.

Since the molecular weight of FTS is 857 (ref. 7), the fractions containing peptides of MW between 0 and 10,000 were further investigated by filtration on Sephadex G-15 to separate the molecules below 1,500 daltons. Unfortunately, they lacked biological activity. Conversely, when 100 μg synthetic FTS (a gift from Hoechst Research Center, rechromatographed and controlled by aminoacid analysis) was added to 2 g of lyophilised 0–10,000 MW ultrafiltrate and filtered on Sephadex G-15 under the same conditions, biological activity was detected in fractions eluting in a larger volume than oxytocin (MW 1,007), indicating the recovery of added FTS.

It was thought, therefore, that FTS could be present in the thymus as a polymer. FTS is a neutral peptide, and so a filtration at acidic pH through a cationic exchanger matrix was carried out (carboxymethyl Sephadex C-25, 2.6×15 cm column, 10 mM acetate buffer at pH 5.0 and 6.3). This step shows that the active material is acidic because it is not bound to the column. Therefore, the chemical properties of this active material seem to be different from those of FTS: its MW is above 1,500 as opposed to 857 for FTS; the active compound is acidic and heat stable (30 min at 70°C) in contrast to FTS, which is a neutral and heat labile peptide⁷. This proves that FTS does not exist as such in the thymus of pig or calf.

The active material isolated from the thymus was further characterised by chromatography on an anionic exchanger (Fig. 1). Only one of the eight fractions obtained exhibited biological activity. The final step involved a molecular sieve filtration (0.9×100 cm column of Sephadex G-25 superfine). The molecular weight of the active peptide was estimated by its elution volume to be 3,000–4,000. Pig and calf thymus gave similar results.

These data show that only one factor active in the T-rosette test can be isolated from the thymus, confirming the work of Goldstein *et al.* on thymosin α 1, a peptide isolated from calf thymus and displaying the same biological activity^{4,10}. As we used non-denaturing methods in contrast to the procedure of earlier workers, it is likely that thymosin α 1 may be the only thymic factor active in the T-rosette test. The chemical properties of the peptide which we have isolated are very similar to those of thymosin α 1¹⁰.

The thymic origin of FTS purified from blood was proposed on the basis of indirect evidence such as loss of activity in the

serum of athymic animals⁸, but it has been questioned by recent biological studies¹¹. The thymic dependence of FTS and its absence in the thymus need to be further discussed in the light of the present results. The aminoacid sequence of FTS⁷ differs from that of thymosin α 1¹⁰, thus excluding the possibility that FTS is a fragment of thymosin α 1. The remaining possibility is that FTS originates in the thymus as hypothetical pro-FTS, lacking biological activity and thus remaining undetectable in the thymus.

N. KELEMEN
F. LASMOLES
G. MILHAUD

INSERM Unité 113,
Hôpital Saint-Antoine,
75571 Paris Cedex XII, France

Received 1 November 1977; accepted 6 January 1978.

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Preferential distribution of surface immunoglobulins on microvilli

Most lymphocyte surface molecules including surface immunoglobulin (s-Ig) are mobile and essentially randomly dispersed in the plane of the membrane (reviewed in ref. 1). It has been recently shown, however, that a spontaneous non-uniform redistribution of surface molecules such as s-Ig (ref. 2), θ antigens³ and concanavalin A receptors¹ can occur in moving cells², or in cells forming spontaneously a uropod^{1,2}. These phenomena, which cannot be explained by considering the membrane purely as a fluid, have been attributed to direct or indirect effects of interactions of membrane proteins with the cytoplasmic structures responsible for locomotion and for the change in cell shape^{1–3}. Here I describe another situation in which a non-uniform distribution of s-Ig is generated during an alteration of the spherical shape of the cell, that is, during formation of microvilli. Such a distribution was not detected in previous transmission immuno-electron microscopy studies, but the microvilli were usually poorly developed. A preferential staining of s-Ig on microvilli has been observed by immunofluorescence⁴, whereas contrasting results (either a uniform⁵ or a non-uniform⁶ distribution) were obtained by scanning electron microscopy using large polyvalent immunolabel particles as markers. I have reinvestigated the point by thin section electron microscopy using a monovalent immunoferritin label in conditions in which microvilli formation, and hence the fraction of membrane on microvilli, is greatly enhanced by ATP deprivation (compare ref. 6). The results show that the label is preferentially concentrated on microvilli.

Mouse BALB/c spleen cells incubated with 10 or 20 mM of sodium azide for 45–60 min at 37°C develop reversibly microvilli up to 5–10 μm long and about 0.1–0.2 μm wide in about 60–80% of the cell. Their formation is inhibited or reversed completely by 10 mM glucose, and partially by cytochalasin B ($10\text{ }\mu\text{g ml}^{-1}$). To study the s-Ig distribution, cells in azide were labelled with monovalent (Fab) or divalent ferritin conjugates of goat (GaMIg-FT), or sheep (Fab-ShaMIg-FT) anti-mouse Ig antibodies.

On all unfixed cells with long microvilli labelled with Fab-GaMIg-FT the ferritin concentration was higher on microvilli than on the cell body (Fig. 1a), although the latter was never completely unlabelled (it could be heavily labelled, however, in cells without microvilli). The ferritin molecules were dispersed

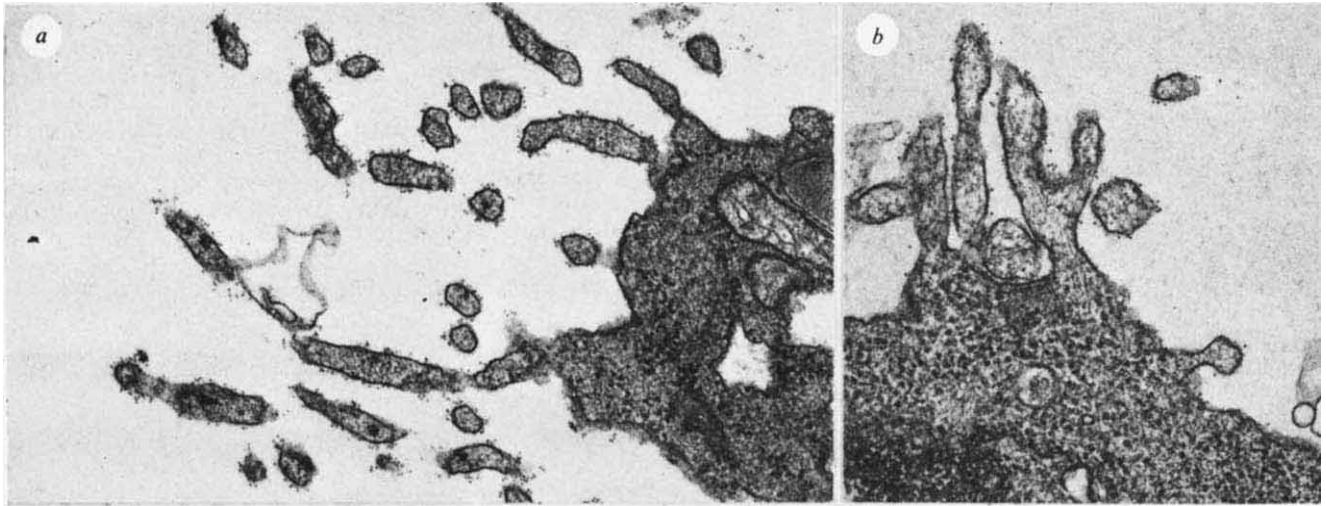


Fig. 1 Distribution of Fab-RaMIg-FT molecules on the microvilli and the body of azide-treated B lymphocyte. *a*, Microvilli surrounding an unfixed B cell (detail). BALB/c spleen cells in physiological saline containing 0.2% bovine serum albumin (BSA), were incubated for 45 min at 37 °C with 10 or 20 mM NaN₃. In these conditions about 60–75% of the cells had long (>3 µm) microvilli (as visualised by staining fixed cells with fluorescein-conjugated concanavalin A) compared with 0–2% in controls without azide. Cells in azide were incubated with the Fab-RaMIg-FT conjugate for 20 min at 37 °C, washed by centrifugation through a 5% BSA layer (always in the presence of azide), and fixed with 3% glutaraldehyde. The conjugate (a gift of Drs S. Avrameas and Th. Ternynck) was prepared by coupling immunoadsorbent-purified anti-mouse Ig antibody Fab fragments with ferritin by the benzoquinone method⁸. The small ferritin clustering observed on these preparations was probably due to the fact that the conjugate, although essentially monomeric and unable to cap, was not freed of some oligomeric aggregates. Bending and distortion in microvilli is caused by centrifugation after fixation. *b*, Prefixed cell (detail). Azide-treated cells were fixed with 2% formaldehyde in 0.12 M phosphate buffer, pH 7.4, for 15 min at 22 °C, centrifuged in 1% formaldehyde (10–15 min), washed three times with buffer and labelled as in (*a*). Magnifications: *a*, ×33,000; *b*, ×43,400.

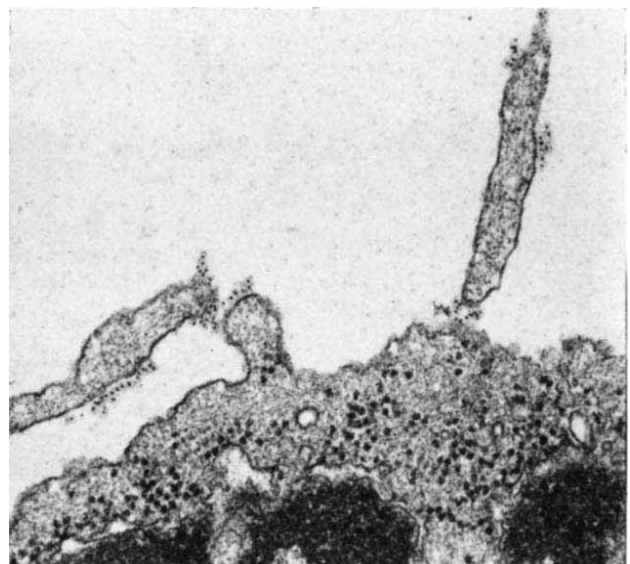
along the entire length of the microvilli either as single molecules or as small clusters (Fig. 1*a*). A distribution essentially identical but more uniform was obtained when the cells were labelled after prefixation with formaldehyde (Fig. 1*b*), although ferritin binding was generally reduced. As indicative values, the ferritin concentration per unit length of membrane in 15 unfixed cells, was 1.6 to 6.2 times higher (mean 3.7) on microvilli than on the cell body. In terms of actual surface density the difference was probably underestimated as the ferritin concentration was measured over the entire undulated profile of the cell body, but only on membrane segments perpendicular to the plane of the membrane on microvilli. To establish whether the Fab antibody/s-Ig complexes on microvilli were still mobile, unfixed Fab-RaMIg-FT-labelled cells were further incubated with an anti-ferritin antibody to cross-link the conjugate. Most of the ferritin was still on microvilli, but was now collected in discrete patches (Fig. 2). The microvilli were often bridged and agglutinated in small bundles by the ligands. Similar patches were obtained when the cells were directly incubated with a divalent GaMIg-FT conjugate.

Although less marked than in azide-treated cells, a preferential redistribution of s-Ig microvilli (two to three times higher than on the cell body; cells prefixed with formaldehyde before labelling) was also observed on normal metabolically active lymphocytes whenever microvilli were well developed, as for example, in stimulated and unstimulated spleen lymphocytes cultured for 24 h with lipopolysaccharide. On the contrary, no preferential distribution of concanavalin A receptors was observed on normal or azide-treated spleen lymphocytes labelled with ferritin-conjugated concanavalin A after fixation with 3% glutaraldehyde. The significance of the latter observation is, however, uncertain, as possible differences in the distribution of individual components might have been blurred by the possible heterogeneity of the labelled receptors, and the general lack of proportionality between the degree of ferritin labelling and receptor density^{7,8}.

These observations suggest that lymphocyte s-Ig molecules tend to leave the cell body and accumulate on microvilli. This spontaneous redistribution, which does not require energy, indicates that the membrane environment in the two membrane compartments is not identical. s-Ig molecules on microvilli are

still mobile and can form patches, so they are not permanently bound to some fixed structural microvillar component (such as molecules, putative connected with the microfilament bundle which runs along the microvillus axis). The reason for the partitioning of the molecules is unclear: there are several possibilities which could explain this. The molecules (or the hypothetical molecular complexes of which they might be a part) could actually bind, but only very weakly and reversibly, to fixed structures preferentially segregated on microvilli. Alternatively, s-Ig solubility could be higher in the microvilli

Fig. 2 Patchy distribution of cross-linked Fab-ShaMIg-FT molecules on microvilli of azide-treated cells. The cells were labelled as in Fig. 1*a*, diluted with buffer and washed by centrifugation through a BSA layer, incubated (10 min, 22 °C) with a diluted (1:100) rabbit anti-ferritin antiserum, and then fixed with glutaraldehyde. With all the conjugates used in these experiments Ig⁻ lymphocytes with, or without microvilli were completely unlabelled. ×42,500.



membrane than in the cell body membrane as a direct, or indirect (for example, lipid-mediated) consequence of differences in the protein composition of the two. For example, less 'solvent space' could become available to s-Ig in the cell body membrane if proteins immobilised by interaction with cytoplasmic structures concentrated preferentially in the latter. A similar explanation has been tentatively suggested for another case of spontaneous redistribution of surface components^{1,3}. Mobile molecules could also become redistributed if this tended to reduce the surface free energy increase induced by the increase in membrane curvature on microvilli.

I thank Miss Ursula Baumgartner for technical assistance.

S. DE PETRIS

Basel Institute for Immunology,
487 Grenzacherstrasse,
Postfach 4005 Basel 5, Switzerland

Received 6 September 1977; accepted 6 January 1978.

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Decreased binding of epidermal growth factor to BALB/c 3T3 mutant cells defective in glycoprotein synthesis

THE surface of animal cells contains a variety of complex macromolecules, some of which modulate cell growth, adhesion and motility. A number of peptide hormones and growth factors influence cellular proliferation and macromolecular synthesis with binding to surface proteins of responsive cells as the first phase of their action¹⁻⁴. The structure and identity of these receptor molecules is a topic of widespread interest. Epidermal growth factor (EGF) is a low molecular weight peptide (MW 6,000) capable of stimulating DNA synthesis and proliferation in epidermal cells⁵, human fibroblasts⁶, and mouse embryo fibroblasts (3T3)⁷. It has also been reported to stimulate the synthesis of a major cell surface glycoprotein (CSP)⁸ and hyaluronic acid⁹ in 3T3 cells and human fibroblasts maintained in low serum. These effects occur at extremely low concentrations of EGF, in the ng ml⁻¹ range, and the first step in this process is the specific binding to surface receptors, followed by rapid internalisation and degradation¹⁰. Pouyssegur and Pastan¹¹ have isolated a nontransformed mutant (AD6) of BALB/c 3T3 cells that has a dramatic decrease in cell surface carbohydrates due to a specific, but partial block in the acetylation of *N*-acetylglucosamine-6-phosphate. This early defect in the biosynthesis of amino sugars leads to incomplete glycosylation of glycoproteins and to decreased exposure of glycoproteins on the cell surface¹². Culturing the cells in the presence of *N*-acetylglucosamine (GlcNAc) bypasses the enzymatic block and results in a restoration of altered surface components¹³. We report here that the mutant AD6 cells show a dramatic decrease in EGF binding, whereas their ability to bind insulin is normal. Our data are consistent with the notion that the receptor for EGF is a glycoprotein.

The mutant cells (AD6) bound about 20% as much ¹²⁵I-EGF as the parental BALB/c cells under standard assay conditions (Table 1). Decreased EGF binding by the mutant cells was observed whether the binding was measured at 37 °C or 4 °C. No appreciable internalisation of EGF occurs at 4 °C (ref. 10), implying that the decrease in binding is not due to the inability of the mutant cells to internalise EGF. For both the normal and mutant cells, the total amount of ¹²⁵I-EGF bound at 4 °C was about 30% of that bound at 37 °C, and this is consistent

with the results of Carpenter and Cohen¹⁰. We also confirmed the results of Todaro *et al.*¹⁴, who found that a cell line transformed by the Kirsten sarcoma virus had dramatically reduced EGF binding.

The possibility remained that damage to EGF during the binding assay could lead to decreased binding or release of substances which could compete with ¹²⁵I-EGF. Therefore, the labelled medium remaining on either AD6 or BALB/c cells at the end of the binding period (45 min) with ¹²⁵I-EGF at 37 °C was placed on untreated BALB/c or AD6 cells. The expected amount of binding occurred when all possible com-

Table 1 Binding of ¹²⁵I-EGF and insulin to mouse fibroblasts

Cell line	Binding temperature and time (min)	Cell density 10 ⁻⁵ cm ⁻²	Sites per cell	Relative binding
BALB/c 3T3	37°- 45	0.84	21,600	1.0
AD6	37°- 45	0.49	4,470	0.21
BALB/c 3T3	4°-120	0.84	8,270	1.0
AD6	4°-120	0.49	1,510	0.18
pg ¹²⁵ I-EGF per 10 ⁶ cells				
BALB/c 3T3	37°- 45	0.46	35.7(3)±1.1	1.0
AD6	37°- 45	0.47	5.7(3)±0.1	0.16
Kirsten				
KA 31 Cl ₁	37°- 45	0.61	5.3(3)±1.4	0.15
BALB/c 3T3 plus tunicamycin	37°- 45	0.70	4.8(3)±0.6	0.13
pg ¹²⁵ I-Insulin per 10 ⁶ cells				
BALB/c 3T3	22°- 60	1.48	1.24(3)±0.1	1.0
AD6	22°- 60	1.14	1.35(3)±0.2	1.1

The cell lines studied were the 3T3 BALB clone C/3 (E. Scolnick, NIH); 3T3 BALB AD6; 3T3 BALB KA31 transformed by Kirsten sarcoma virus (Dr E. Scolnick, NIH). The cells were grown in Dulbecco-Vogt's modified Eagle's medium supplemented with 10% foetal calf serum in 35-mm plastic tissue culture dishes (Costar) under 95% air-5% CO₂ humidified atmosphere at 37 °C. Unless stated otherwise, the cells were plated at 2 × 10⁴ to 1 × 10⁵ cells per dish (0.2-1 × 10⁴ cells cm⁻²) in 5-ml medium, fed after 24 h and used after 96 h. In some experiments, *N*-acetylglucosamine (Sigma), or tunicamycin (gift of G. Tamura) were included in the medium. For binding studies, the cells were not detached from the culture dishes, because it was found that nonspecific binding of ¹²⁵I-EGF or ¹²⁵I-Insulin to the plastic dish was negligible. For rinsing of cultures before and after the actual binding and also for the binding itself, the following medium was used: Dulbecco-Vogt's modified Eagle's medium (DME) containing 1 mg ml⁻¹ bovine serum albumin (Sigma), penicillin and streptomycin at 50 units or µg ml⁻¹, and 25 mM Hepes (*N*-2-hydroxyethyl piperazine-*N*'-2-ethanesulphonic acid) at pH 7.4. Before binding, the cultures were gently washed twice at 25 °C with 2 ml of binding media, and 1 ml of binding media was then added to each culture. Those cultures that were to be used for determination of nonspecific binding received 20-30 µl (2-3 µg) of mouse EGF (gift of S. Cohen). Following this, 5-100 µl of ¹²⁵I-EGF (mouse) or ¹²⁵I-insulin were added to each culture, except those used for determination of cell number. The ¹²⁵I-EGF was supplied by Drs Ann Dockstetter and Paul Kelley of Collaborative Research, at a specific activity of 30-50 µCi µg⁻¹ and the ¹²⁵I-insulin (150 µCi µg⁻¹), supplied by Dr Jesse Roth of the Diabetes Branch, NIH. For most experiments, they were added at 0.1-1 ng ml⁻¹. The cultures were then incubated at 37 °C for 30-45 min in a 95% air-5% CO₂ atmosphere or at 4 °C for 2 h. The binding media were then rapidly aspirated and the cells gently washed four times with binding media at 4 °C. The cells were solubilised for 20 min at 37 °C in 1.0 ml of lysing buffer composed of 10 mM Tris-HCl, pH 7.4, 1 mM EDTA and 0.5% sodium dodecyl sulphate and the contents transferred to a counting vial with a siliconised Pasteur pipette. Each dish was then rinsed with 1 ml of lysing buffer. The contents were transferred to a counting vial containing 15 ml of the Hydromix scintillation fluid. The amount of radioactivity was measured in a liquid scintillation spectrometer. Nonspecific binding was always less than 5% of the total counts bound and was subtracted from all reported values. Generally, two to three Costar trays, each with six 35-mm dishes, were processed at a time, with three dishes per tray being used for total binding, one for nonspecific binding and two for cell counts. The latter cells were released by trypsinisation (0.25% w/v) for 15 min at 37 °C. An equal volume of growth medium containing 10% foetal calf serum at 4 °C was added and the cell number determined in an electronic particle counter (Coulter).

binations were tested (results not shown). Thus, degradation of EGF or endogenous release of EGF-like substances from the cells during the binding period does not account for the diminished capacity of AD6 cells to bind EGF.

BALB/c cells also possess receptors for insulin. The results of Table 1 indicate that the mutant cells are not defective in binding of ^{125}I -insulin under conditions which show maximal difference between BALB/c and AD6 cells in binding of ^{125}I -EGF.

The amount of EGF bound by BALB/c cells is strongly dependent on cell density, whereas the amount of EGF bound to AD6 cells is relatively unaffected by this (Fig. 1). BALB/c 3T3 cells increase the number of EGF binding sites by 10-fold between 2 and 7×10^4 cells cm^{-2} , whereas this increase is only 2-fold for AD6 cells between the same densities. These densities range from sparse to confluent. Identical results were obtained when the cells were plated at 1×10^4 cells cm^{-2} , and binding measured daily over the next 4 d. The dependence on the amount of EGF bound as a function of ^{125}I -EGF added is illustrated in Fig. 2. Saturable binding was found in both BALB/c and AD6 cells, with half maximal binding occurring at 4 ng ml^{-1} or $6 \times 10^{-10} \text{ M}$.

Pouyssegur *et al.*¹³ showed that the addition of 10 mM GlcNAc to the culture media of AD6 cells for 3–7 d restored their morphology to normal and also corrected their defect in glycoprotein synthesis. Therefore, we treated AD6 and normal cells with varying amounts of GlcNAc (3 – 9 mM) and measured EGF binding at the end of 4 d. The results shown in Fig. 3 indicate that GlcNAc at 3 – 7 mM caused increased EGF binding to AD6 but decreased the binding of EGF to wild-type cells. However, EGF binding was not fully restored to the level observed in the parental cell type, and at 9 mM GlcNAc, the binding of EGF to AD6 was slightly reduced compared to that at 7 mM . We believe the failure of GlcNAc feeding to restore EGF binding completely to normal reflects alterations in the pattern of glycosylation induced by GlcNAc feeding or a metabolic product derived from it, because the binding of EGF to the normal cells began to fall at quite low concentrations of GlcNAc (Fig. 3).

Fig. 1 Binding of ^{125}I -EGF as a function of cell growth. The cells were initially seeded at 10^3 – 2×10^4 cells cm^{-2} , the media changed the following day, and binding assayed on the fourth day. Each value represents the mean of three determinations $\pm$ s.e.m. —, BALB/c; --- AD6.

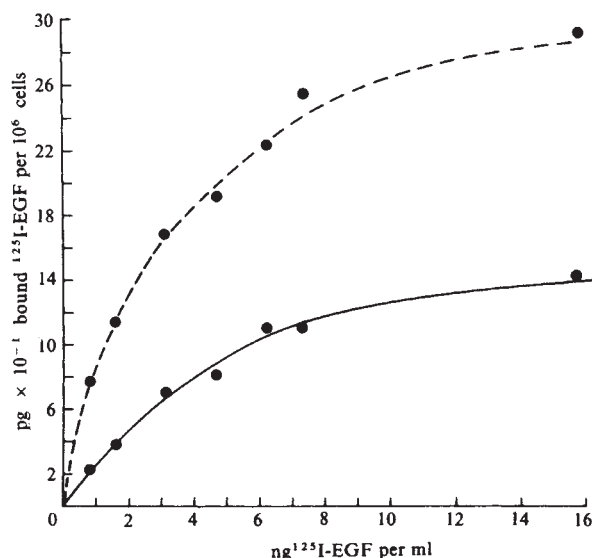
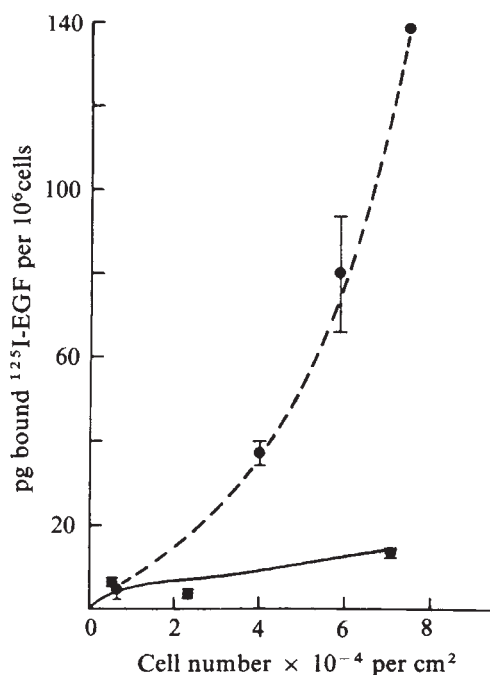


Fig. 2 Characterisation of ^{125}I -EGF binding to mouse fibroblasts. The cells were initially seeded at 10^4 cells cm^{-2} and on the fourth day binding was determined as described in Table 1. The ^{125}I -EGF was at a concentration of $0.7 \text{ ng per } 5 \mu\text{l}$, and $5 \mu\text{l}$ – $100 \mu\text{l}$ were added per ml to initiate the binding. BALB/c 3T3 (---) cells were at 6.0 and AD6 (—) cells at 8.0×10^4 cells cm^{-2} . The values represent the mean of two determinations.

The defect in the acetylation of glucosamine-6-phosphate by AD6 cells probably also results in decreased formation of complex glycolipids, and the possibility exists that this may contribute to decreased EGF binding. In order to test this possibility, EGF binding was examined in normal and tunicamycin-treated 3T3 cells. Tunicamycin is an antibiotic which specifically inhibits the dolichol-mediated glycosylation of many glycoproteins but not glycolipid glycosylation or synthesis^{15,16}. Exposure of 3T3 cells to 50 ng ml^{-1} of tunicamycin resulted in a dramatic decrease in protein glycosylation¹⁵ and reduction in EGF binding similar to that observed in AD6 cells (Table 1).

EGF stimulates proliferation of various epidermal and epithelial tissues *in vivo* and *in vitro*^{2,5}. It is also a potent mitogen (1 – 10 ng ml^{-1}) for human glial cells¹⁷. Although a specific hormonal role for EGF has not been defined, this polypeptide may serve as a model for serum substances which promote cell growth.

The most likely explanation for the observed low EGF binding to AD6 is that the EGF receptor is a glycoprotein and that in the mutant cell the EGF receptor is either not exposed at the cell surface or is exposed but the carbohydrate component(s) of the EGF receptor necessary for EGF binding are lacking. Alternatively, the EGF receptor itself may not be a glycoprotein, but may require the presence of normal surface glycoproteins in close proximity to interact with EGF in a stereospecific manner to facilitate normal binding. There is evidence, however, which suggests that the EGF receptor is a glycoprotein. Hock and Hollenberg¹⁸ and Carpenter and Cohen¹⁹ have found that various lectins reduce EGF binding to placental membranes and human fibroblasts, respectively. Although the mutant AD6 does not exhibit the growth properties of transformed cells, it does possess the morphological phenotype and other features commonly associated with transformed cells. These include a rounded shape, numerous surface microvilli, low adhesion to substratum, and increased agglutinability by concanavalin A. It is striking, therefore, that AD6, like cells transformed by murine sarcoma virus (MSV) and feline sarcoma virus (FeSV), has diminished EGF binding, but no apparent alteration in binding of insulin or MSA (multiplication-stimulating activity). It has been reported, however, that a variety of viral or chemically transformed cells have somewhat decreased binding of insulin²⁰. Todaro *et al.*^{14,21} have observed that EGF binding, but not MSA bind-

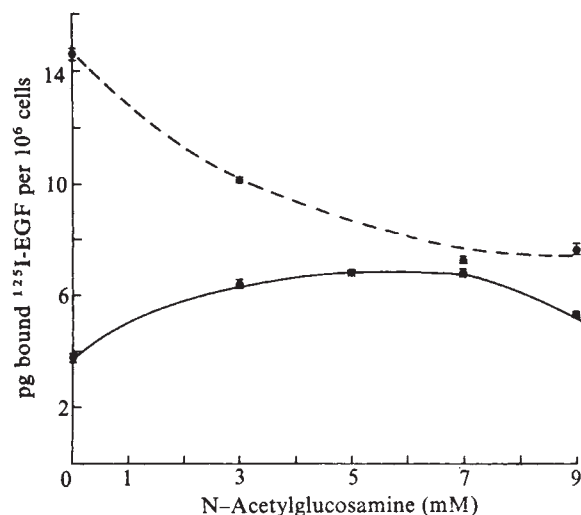


Fig. 3 Binding of ^{125}I -EGF to BALB/c 3T3 (---) and AD6 (—) cells cultured with *N*-acetylglucosamine. Cells were initially seeded at 10^4 cells cm^{-2} with or without the indicated concentration of GlcNAc and assayed on the fourth day. The values are the mean of three determinations $\pm$ s.e.m.

ing, is decreased in MSV and FeSV transformed cells, but not in other types of transformed cells. These authors have suggested that this low binding might be due to an EGF-like factor made by these cells that competes with authentic ^{125}I -EGF for binding to surface receptors. Our results with AD6 raise the possibility that MSV and FeSV transformed cells could have an abnormality in glycoprotein synthesis contributing to the alteration in EGF binding. It has been reported²² that the glycoproteins in many types of transformed cells possess structural alterations, although characteristic or specific defects in different types of transformed cells have not been identified. Nevertheless, the possible relationship of an alteration in glycoprotein synthesis to the low binding of EGF observed in MSV and FeSV transformed cells seems to warrant further investigation. Preliminary experiments suggest that the decreased binding of EGF to AD6 cells causes a diminution in the proliferative response to EGF compared to BALB/c 3T3 cells.

We thank Elizabeth Lovelace and Webster C. Leyshon for technical assistance.

ROBERT M. PRATT
IRA PASTAN

Laboratory of Developmental Biology and Anomalies,
National Institute of Dental Research
and

Laboratory of Molecular Biology,
Division of Cancer Biology and Diagnosis,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 31 October; accepted 19 December 1977.

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Microprobe measurement of Na, K and Cl concentration profiles in epithelial cells and intercellular spaces of rabbit ileum

THE absorption of many solutes and water by the intestinal mucosa is linked passively to an active transport of Na^+ to produce an absorbate which is isotonic with the capillary plasma^{1,2}. Since Curran³ first proposed a double membrane model with a hypertonic compartment within the tissue, it has generally been accepted that the primary sites of Na^+ transport^{4,5} and solute–water coupling are the lateral intercellular spaces (LIS) within the epithelium (for reviews see refs 6 and 7). Several theoretical models have been proposed to explain how the secretion of Na^+ into LIS generates flow of water, but the sites within the tissue where the final isotonicity of the absorbate is achieved remain controversial^{8–15}. This is chiefly because it has not been possible directly to measure the profiles of ionic concentrations in the LIS and surrounding tissue compartments. Indirect evidence has suggested that the extracellular spaces in gall bladder¹⁶, feline intestine¹⁷ and rabbit ileum¹⁸ contain higher concentrations than those found in external bathing solutions. We have now used electron microprobe X-ray analysis^{19–22} of frozen-hydrated sections to measure the concentration profiles of Na, K, Cl, Ca, S and P in the mucosal tissue of rabbit ileum. The results establish the hypertonicity of the fluid in the LIS and the concentration gradients of Na, K and Cl in the cells and in the LIS.

In Fig. 1 the average concentrations of Na, K and Cl in normal controls are compared with the concentrations in ouabain-treated tissue. As expected, ouabain increases the cytoplasmic Na and Cl and decreases K, compared with control tissue. With 10 mM galactose present in the bathing solution, the average concentration of cytoplasmic Na in control tissue (36 ± 3 mM) was less than that previously estimated by routine analytical procedures (61 ± 4 mM) (ref. 23), but was in accord with that found by compartmental analysis (43 ± 9 mM) (ref. 18). Similarly, cytoplasmic K was close to values known by other methods^{18,23} but Cl was somewhat lower²⁴, although the incubation conditions used for Cl determination²⁴ were different from ours. Furthermore, the microprobe measurements in Figs 1–3 are given as mM per kg wet weight. If all the ions are dissolved in tissue water, then the free concentrations expressed as mM per kg H_2O will be higher²¹. Thus, in general, our measurements of average intracellular elemental concentrations conform to expectations from previous physiological studies.

The values given in Fig. 1 mask the local differences in concentrations of Na, K and Cl in the cytoplasm. In control tissue, the concentrations of Na and Cl in the organelle-rich cytoplasm of the cell core decreased as the finely focused probe was moved from the apical to the nuclear region. The magnitude and direction of these gradients are similar to the gradients of Na^+ and Cl^- activities observed with ion-selective microelectrodes by Zeuthen²⁵. In our microprobe measurements, the values refer to the elemental concentrations (bound and unbound), and the image details do not afford a clear resolution of the organelles from the cytosol. However, we have no evidence to support the biochemical observations²⁶ that the concentration of Na is far greater in the nucleus than in the cytoplasm.

Our study reveals an approximately $0.5\text{-}\mu\text{m}$ wide zone of peripheral cytoplasm immediately adjacent to the cell membrane

and including the terminal web area (Fig. 2), which has less S, more P and much less dry mass (14% of wet mass) than the cytoplasm in the cell core (25% of wet mass). This peripheral zone of cytoplasm constitutes about 10–20% of the cell volume,

shows no significant gradients of ions, but has a much higher average concentration of K than that in cell core. Assuming no ion binding and electroneutrality, it seems to constitute a compartment with osmolality ($2 [K + Na]$) intermediate

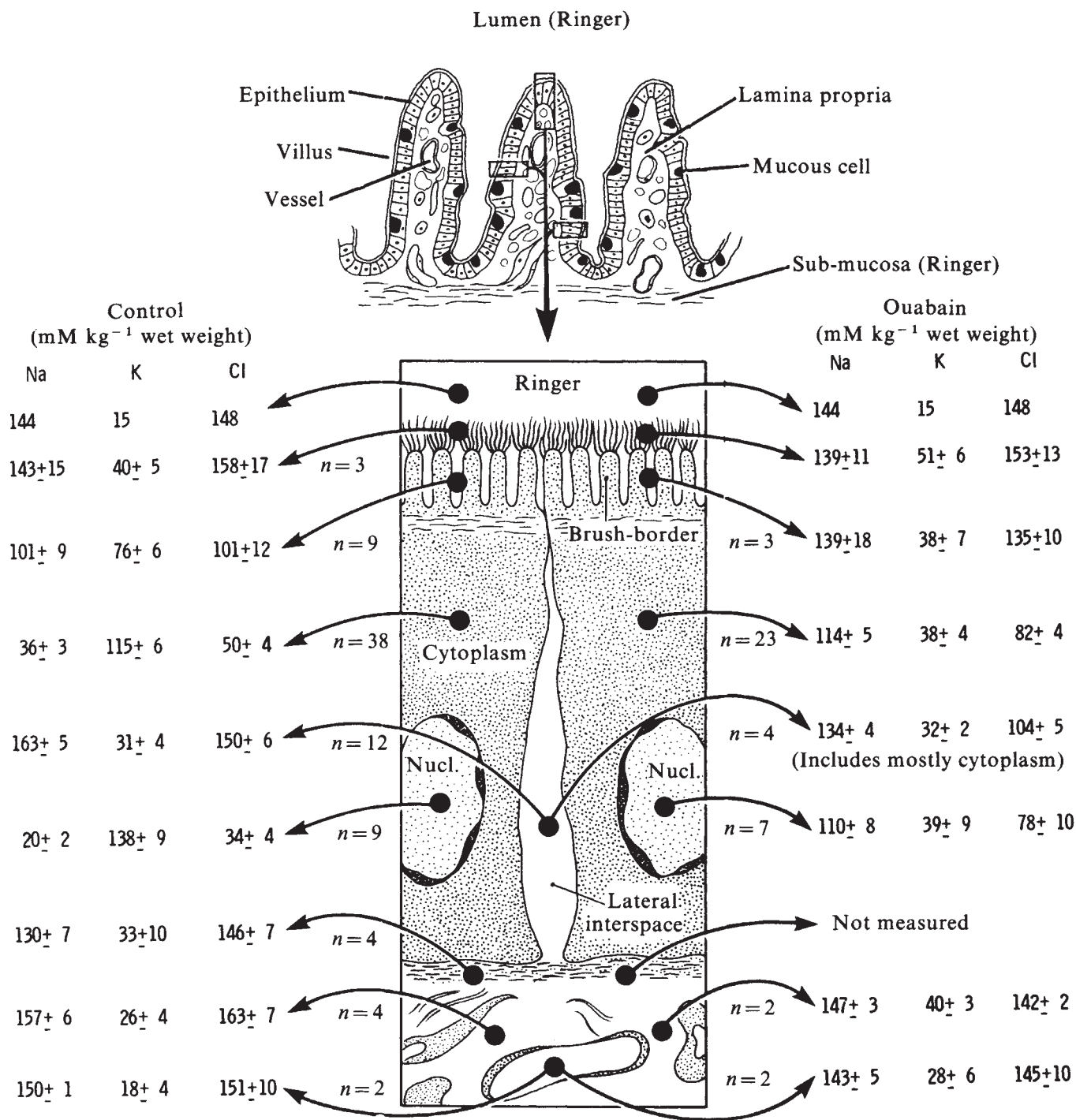


Fig. 1 Average concentrations (mean $\pm$ s.e.m.) of Na, K and Cl in the villus of rabbit ileum as measured by electron microprobe X-ray analysis of frozen-hydrated sections. In addition to the visual recognition, the lateral intercellular spaces were positively identified by their very low (≤ 20 mM) P and S levels as compared with the adjacent cytoplasm (P $\sim$ 200 mM, S $\sim$ 50 mM). The statistical analysis using Student's *t* test (unpaired) showed that all the differences in mean values between control and ouabain-treated tissue were highly significant ($P < 0.001$). To prepare the tissue for microanalysis, the serosal and external muscle layers were stripped off the rabbit ileum. Sheets of mucosa were mounted in a multiple chamber¹⁸ and incubated for 40 min at 37 °C in physiological saline¹⁸ continuously gassed with 95% O₂ + 5% CO₂. Galactose (10 mM) was added to the saline to stimulate active fluid transport³³ and 10% Dextran (molecular weight 400,000) was added to prevent later ice-crystal damage and to improve sectioning¹⁸. Parallel preparations were set up with 0.1 mM ouabain added to the saline to block Na transport. At the end of incubation the tissue sheets were transferred to the temperature-regulated (37 °C) dissection chamber of a stereomicroscope. Pieces of epithelium of 1 mm² containing 5–10 villae were cut out with sharp razor blades, mounted on copper pins and immediately quench-frozen in melting Freon-13 (monochlorotrifluoromethane) at -180 °C. 1- μ m thick sections of the frozen tissue were cut at -80 °C, transferred to a JEOL JXA-50A microanalyser and analysed while on a cold stage maintained at -170 °C. Details of sample preparation, quantification of X-ray data and the method for the estimation of water in analysed microvolumes of the sections have been described elsewhere^{19,21}.

between the bathing medium and the fluid in the LIS^{27,28}. It is conceivable that this peripheral zone acts as a channel in which ions are more mobile and along which Na⁺ and Cl⁻ are more rapidly conducted by an actomyosin system, like that reported in the cortical cytoplasm of non-muscle cells^{29,30}. The cell core, with its high concentration of membranous organelles, may thus form a relatively unstirred compartment with slower rates of diffusion⁷. This may in part account for the observed inhomogeneities of ions within the cytoplasm. Ouabain abolishes both the axial and the radial differences in the local ionic concentrations in the cytoplasm, indicating that the observed gradients in control tissue are not due to either the artefacts of tissue preparation or the stable binding of ions.

We found that the average concentrations of Na and K in the LIS were significantly higher than the concentrations in the external bathing solutions and in the capillary fluid ($P < 0.05$ for Na; $P < 0.025$ for K). Detailed analyses of Na, K and Cl distributions along the LIS and into the lamina propria (Figs 2 and 3) indicate distinct gradients, with a plateau about halfway along the channel where, assuming electroneutrality, the fluid may be as much as 100 mOsm hypertonic to the bathing solution. The measurements in Fig. 3 are from a single LIS, so that the relative values are established without scatter from specimen or preparative variability. The concentration profile in the junctional half of the LIS shows a much steeper gradient within

the wider region of the channel (Fig. 2; Na gradient 151–177: $P < 0.025$) than the one (144–151: $P < 0.05$) across the narrow junctional channel. If this were entirely due to an outward diffusion of Na across the leaky junction, then the diffusion coefficient of Na across the junction would exceed that in the wide interspace by at least three orders of magnitude. We interpret the concentration profiles in Figs 2 and 3 to suggest a considerable osmotic flow of water across the junction into the LIS. If the zonulae occludentes were impermeable to fluid, the mass flow at this end of the LIS would be minimal and the concentration maximal, as in the model of Diamond and Bossert³¹. In ouabain-treated tissue, the LIS collapsed and could not be resolved from the cytoplasm. However, the concentration of K but not of Na and Cl in the lamina propria remained higher than in the bathing solution (Figs 1 and 2), as previously found by compartmental analysis¹⁸.

The original elemental distributions may have been affected by diffusion artefacts during specimen preparation, especially at the stage of quench-freezing, but diffusion and the limited spatial resolution of microprobe analysis (here about 300 nm)¹⁹ can only affect the results by 'smoothing out' the gradients in the control tissue. The artefacts could not have generated gradients. The technical limitations therefore cannot invalidate the concentration gradients revealed by our data, although they may reduce the magnitude of such gradients.

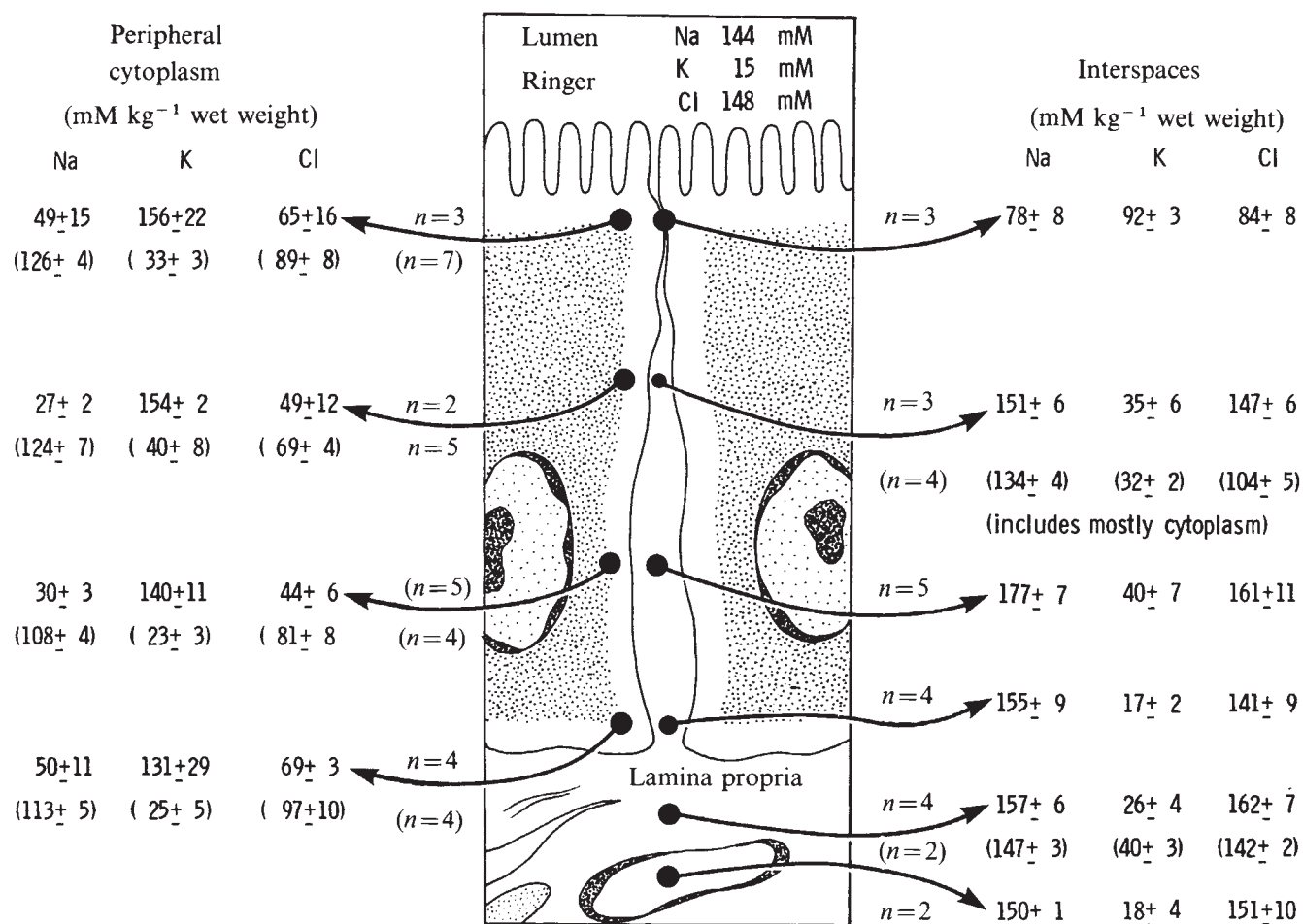


Fig. 2 Concentration profiles of Na, K and Cl (mean $\pm$ s.e.m.) in the lateral intercellular space are compared with those in the adjacent zone of peripheral cytoplasm. The values in parentheses are from the tissue treated with 0.1 mM ouabain for 45–60 min. The statistical significance of the mean values for Na and K in the LIS were determined by one-way analysis of variance using the F test of Fisher and Yates. The P values for Na are: base relative to middle, $P < 0.025$, middle relative to apical opening, $P < 0.01$, all LIS Na relative to Na outside, $P < 0.05$; and for K: base relative to middle, $P < 0.001$, middle relative to opening, $P < 0.01$, all LIS K relative to K outside, $P < 0.025$. The measured dry mass fractions are about 6% of the wet mass in the LIS, about 10% in the lamina propria and about 14% in the peripheral cytoplasm. Assuming that the measured elements are entirely dissolved in the aqueous fraction, one can obtain the concentrations of Na, K and Cl in mM l⁻¹ H₂O by multiplying the present values by 1.06 $\times$ for the LIS, by 1.11 $\times$ for the lamina propria and by 1.19 $\times$ for the peripheral cytoplasm.

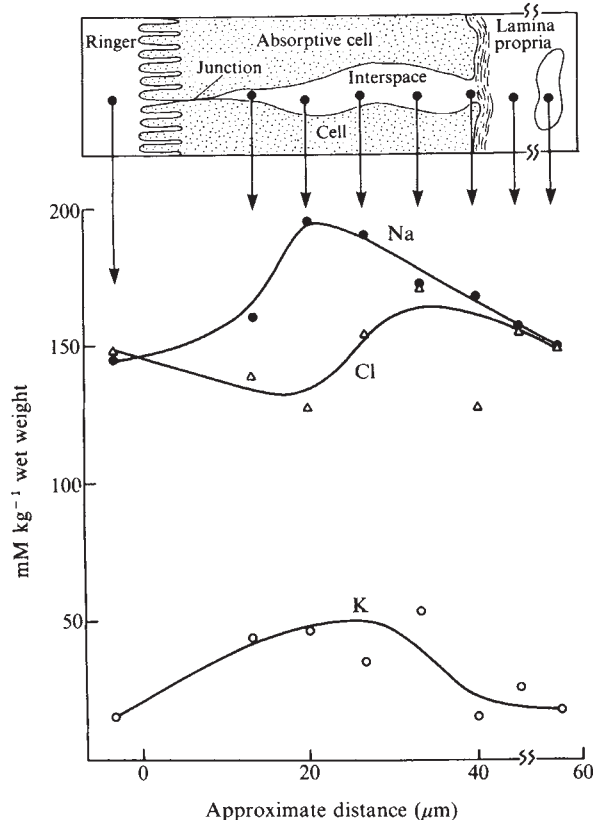


Fig. 3 Concentration profiles of Na, K and Cl along a single intercellular space, measured by locating a fine electron probe (diameter about 100 nm) in steps of about 5 μm along a linear axis. The smooth curves are drawn by eye through the experimental points. The individual values for the concentrations of all three ions in the LIS are not significantly different from the mean values at the corresponding sites in Fig. 2.

From our data, we conclude that during the galactose-induced fluid absorption in rabbit ileum the lateral intercellular spaces are hypertonic and form the primary sites of solute-water coupling. A narrow zone of peripheral cytoplasm in columnar epithelial cells constitutes a relatively homogeneous compartment, with osmotic concentration intermediate between the mucosal bath and the LIS; it may form the main route for the transcellular movement of fluid and determine the osmolarity of the fluid emerging from the LIS. The osmotic equilibration of the absorbate in LIS involves appreciable flow of water through the cell junction and may only be completed in the lamina propria. The observed concentration profiles of Na, K and Cl in and around the LIS are not consistent with the standing gradient hypothesis³¹, but resemble those predicted by other recent models based on local osmosis^{11,12,32}. Detailed results and full implications of the present study will be published elsewhere²⁸.

The Biological Microprobe Laboratory is supported by a grant from the SRC. We thank Kate Barber and A. J. Burgess, M. J. Day, J. W. Rodford and P. G. Taylor for technical assistance.

BRIJ L. GUPTA
T. A. HALL

Department of Zoology,
Biological Microprobe Laboratory,
Cambridge, UK

R. J. NAFTALIN

Department of Physiology,
King's College,
Strand, London, UK

Received 1 August 1977; accepted 10 January 1978.

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Opiate tolerance and dependence induced *in vitro* in single myenteric neurones

OPIATES inhibit the firing of neurones in the myenteric plexus of the guinea pig ileum in a manner which is strikingly similar to their effects on many central neurones. This action on single neurone firing has been studied in detail¹⁻³; it occurs at low concentrations of opiates¹⁻³ and opioid peptides⁴, it is mimicked by the (—) but not by the (+)-isomer of optical enantiomers, and it is reversed or prevented by the specific antagonist naloxone¹⁻⁴. Naloxone has no effect on the firing rate of myenteric neurones removed from normal animals; however, it does cause a marked increase in the rate of firing of myenteric neurones taken from guinea pigs which have previously been made physically dependent on morphine⁵. Previous studies have shown that some signs of opiate tolerance and dependence can be induced in the guinea pig ileum both *in vitro*^{6,7} and *in vivo*^{8,9}. In the present experiments, we have induced tolerance to the effects of opiates by exposing myenteric neurones to agonists *in vitro* for periods of about 24 h. Such neurones also showed manifestations of dependence on the presence of the opiate, in that their rate of firing increased markedly when the agonist was removed or when an antagonist was introduced. The tolerance and dependence were induced only by exposure to the (—)-isomer of enantiomeric agonists, and the increase in firing was precipitated only by the (—)-isomer of enantiomeric antagonists.

Pieces of ileum several cm in length were removed from newly killed guinea pigs and placed in a Krebs solution. This was changed several times during a period of about 24 h. The composition of the Krebs solution has been described³; otherwise the conditions of incubation were pH 7.4, gassed with 95% O₂/5% CO₂, 21-24 °C. After this period of incubation, extracellular recordings were made from single neurones of the myenteric ganglia as previously described³. Such incubation of the intestine for 24 h before single unit recording had no marked effects on the spontaneous firing which was observed. Morphine (or normorphine) (1-100 nM) prevented the spike firing (Fig. 1) and this is very similar to the effects of morphine

on neurones which have not been incubated¹⁻³. Spike firing was completely suppressed by high concentrations of normorphine (1 μ M) and remained inhibited throughout the period of drug application (Fig. 1).

Tissue from 14 animals was kept in Krebs solution containing morphine (1 μ M) for 24 h before preparing the ganglia for extracellular recording. The neurones were continuously exposed to morphine (1 μ M) throughout the period of incubation and the subsequent period of extracellular recording. The rate of firing of these neurones did not appear to be different from those which had been incubated without morphine, but the firing pattern was generally more erratic and distinct bursts of spiking were more frequently observed than in control incubated tissue. Marked tolerance was developed to the inhibitory effect of morphine on neuronal firing. Increasing the morphine concentration to 10 or 30 μ M was usually without effect on the firing rate. There was a clear increase in the firing rate of 9 out of 14 neurones when the solution was changed to one which differed only in its lack of morphine. Similar but more marked increases in firing were observed when the perfusing solution was changed to one which contained both morphine and naloxone (Fig. 2). The firing caused by naloxone was dramatic; it began immediately upon the initiation of naloxone treatment and spike frequency increased 3- to 10-fold. The naloxone effect occurred in 19 out of 26 units, it was concentration related (10 nM–1 μ M), and it usually outlasted the period of application (2–3 min).

To test whether this action of naloxone was due to the displacement of the opiate agonist from the receptor, we used optical enantiomers of another antagonist, 5,9 α -diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan hydrochloride. The (–)-isomer (Mr2266) produced an increase in firing exactly similar to that caused by naloxone, but the (+)-isomer (Mr2267) was without effect (Fig. 2). In several experiments, a single exposure to antagonist induced longlasting rises and falls in the firing rate, with a periodicity of 2–5 min, and continuing for 10–20 min after the antagonist was washed out.

In order to test whether the effects of incubation were the consequences of the prolonged occupation of the opiate receptor, tissues were incubated either with the opiate agonist levorphanol or with its inactive enantiomer dextrorphan. Recordings were made from 16 units (nine animals) which had been incubated for about 24 h in levorphanol (100 nM). Nine out of 10 units tested were tolerant to the inhibitory effect on spike firing normally displayed by levorphanol (300 nM–1 μ M). Cross tolerance to inhibition by morphine was also apparent. The firing of these units was markedly increased by changing the perfusing solution to one which was free of levorphanol. Similarly, the antagonists Mr2266 or naloxone

Fig. 1 Inhibition of firing of a myenteric neurone by normorphine. Before the beginning of this recording (time zero), this neurone had been incubated in Krebs solution for 24 h. During the periods indicated by the solid bars, the solution which perfused the tissue contained normorphine in the concentration indicated (nM). This neurone was one of the most sensitive to inhibition by morphine which we have observed. Following prolonged (30 min) exposure to a high concentration of normorphine (1 μ M), the neurone became somewhat less sensitive to the subsequent effect of the lower concentrations.

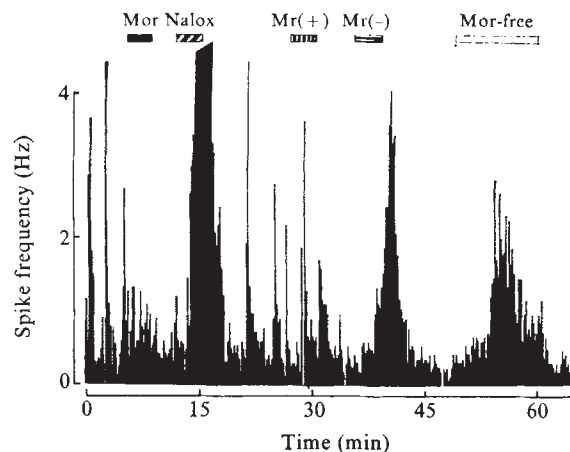
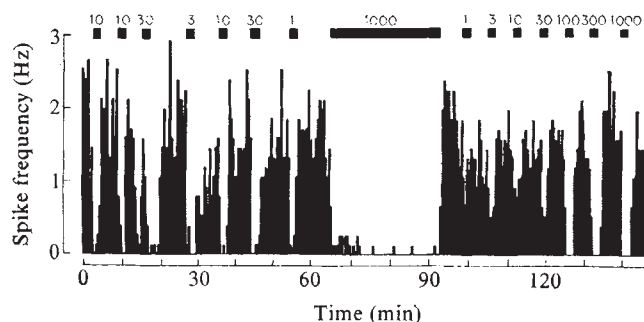


Fig. 2 Effect on a myenteric neurone of prolonged exposure to morphine in conditions of synaptic transmission blockade. Before beginning the recording (time zero), this neurone had been exposed continuously to a solution containing morphine (1 μ M), hyoscine (1 μ M) and hexamethonium (300 μ M). This solution continued to perfuse the tissue throughout the period of recording except for the time indicated by the open bar, when it contained only hyoscine and hexamethonium. During the periods indicated by the other bars, the perfusing solution contained additionally morphine (to a final concentration of 10 μ M, Mor), naloxone (100 nM, Nalox), Mr2267 (100 nM, Mr(+)) or Mr2266 (100 nM, Mr(–)). Note that the neurone was tolerant in that a very high concentration of morphine no longer depressed its firing (see Fig. 1). Naloxone caused a pronounced increase in firing (this reached 8–10 Hz), Mr2267 had no clear effect on the firing rate, but its (–)-enantiomer, Mr2266, caused a marked excitation. This neurone was also excited simply by removing the morphine from the perfusing solution. In this and other experiments, second and subsequent exposures to antagonists did not cause as marked an excitation as did the first application.

produced clear increases in spike firing in 14 of 16 experiments. Neurones which had been incubated in dextrorphan (100 nM) for 24 h were still sensitive to inhibition by morphine, and their firing rates were not increased by antagonists or by dextrorphan-free solutions.

The activity recorded in these experiments is not related to any synaptic input to the cells^{2,3}. The question arose as to whether the development of opiate tolerance and dependence required an intact and functioning neuronal network. Tissue from six animals was incubated in a solution which contained morphine (1 μ M), hyoscine (1 μ M) and hexamethonium (300 μ M). The concentration of hexamethonium used blocks all the synaptic potentials recorded intracellularly from myenteric plexus neurones¹⁰; the hyoscine was sufficient to block transmission from nerve to muscle^{11,12} and thereby prevent neuronal activity which might be secondary to the muscle movements brought about by resting release of acetylcholine. Extracellular recording from neurones so treated gave results similar to those from neurones which had been incubated with morphine alone. All nine units tested were not inhibited by morphine (10 μ M), 13 of 16 units tested were excited by antagonists (Fig. 2) and the firing rate of each of two units tested was increased by applying a solution which was morphine-free but which still contained hexamethonium and hyoscine (Fig. 2).

These experiments indicate that prolonged exposure to opiates of neurones *in vitro* produces tolerance to the normal inhibitory effects which these agents have on cell firing. Opiate dependence is clearly demonstrable by the increase in firing which occurs when the opiate is withdrawn; the more marked effects brought about by antagonists may be presumed to simulate precipitated abstinence. The apparent ease with which tolerance and dependence can be induced in single neurones, and the fact that these changes are not dependent on trans-synaptic influences, are of particular interest. In terms of the action of opiates, the parallels between myenteric neurones and the central nervous system are close and numerous^{2,3,13}.

The analogies between the present findings and certain clinical manifestations of tolerance and dependence are also sufficiently strong to encourage further investigation of the mechanism of the clinical phenomena at the single neurone level.

We thank Drs E. L. May and H. Merz, Endo Laboratories and Hoffman La Roche, for their respective gifts of normorphine, benzomorphan antagonists, naloxone and levorphanol. The work was supported by a US PHS grant.

R. ALAN NORTH
PETER J. KARRAS

Neurophysiology Laboratory,
Department of Pharmacology,
Loyola University Stritch School of Medicine,
Maywood, Illinois 60153

Received 2 November 1977; accepted 3 January 1978.

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Ethanol tolerance and the *Adh* polymorphism in a natural population of *Drosophila melanogaster*

A DETAILED understanding of the balanced selective forces maintaining the alcohol dehydrogenase (*Adh*) polymorphism of *Drosophila melanogaster* will come about when the properties of the enzymatic products of the genotypes can be directly related to selective forces acting on the phenotypes. This approach has been discussed by Clarke¹, but it was Gibson² who first probed the *Adh* locus in this way when he successfully selected for a specific allele using high levels of ethanol. Enzyme activity of *Adh^F/Adh^F*, *Adh^F/Adh^S* and *Adh^S/Adh^S* genotypes on specific alcohol substrates can generally be related to differential survival and changes in gene frequency in population cages^{1–8}. Briscoe, Robertson and Malpica³ showed a higher *Adh^F* frequency in a Spanish wine cellar population than at a neighbouring site and explained this by the relative ethanol resistance of adult *Adh* phenotypes. In a study of an Australian winery population McKenzie and Parsons⁹ also observed differences in ethanol tolerance between the cellar and peripheral sections of the population but they found no correlation between tolerance and *Adh* frequencies. We now report a more intensive study of this population where samples have been taken from inside and outside the cellar during both vintage and non-vintage periods over two years. In this population a different adaptive system is operating. Unlike Briscoe *et al.*, we found no

relationship between the distributions of tolerance and of *Adh* phenotypes although tolerance can be directly related to the selective effect of alcohol in the adult environment.

The 'Chateau Tahbilk' population has been studied in considerable detail^{9–13}. During the vintage period alcohol fumes from the fermentation area above the cellar affect the distribution of *D. melanogaster* such that the species moves towards the cellar from the surrounding countryside. At other times, when alcohol fumes are not apparent, the direction of movement of the outside cellar section of the population is nearly random¹¹. Effective migration between sections of the population is likely to be minimal at times other than vintage but even during the vintage period, when gene flow is significant, ethanol tolerance differences between the two sections of the population persist¹².

In this study the area outside the cellar was divided into four (Table 1). The areas were chosen to cover a trapping grid of the original collection and to ensure that reasonable numbers could be collected in each area¹¹. Population samples were taken from each area and from the cellar immediately before, during and immediately after the 1975 and 1976 vintage periods.

At least 10 iso-female strains were started from each area for each collection. All adults collected were scored for *Adh* phenotype using 'Cellolog' electrophoresis. Tolerance of iso-female strains was ascertained by scoring the proportion of second laboratory generation larvae that reach the adult stage after development on medium supplemented with 9% ethanol. This larval tolerance measure was significantly correlated ($r = 0.73$, $P < 0.001$) with the survival of adults of the same strain kept in vials saturated with ethanol fumes. The 50 strains used for this comparison were derived from all areas of the population.

The trends observed during both vintage periods were similar, as were those for the non-vintage samples. The *Adh^F* frequencies and the mean ethanol tolerances (Table 1) have therefore been determined from pooled data. Genotype frequencies for the *Adh* locus consistently fitted Hardy–Weinberg expectations. Only one sample of the 30 comparisons differed significantly ($P < 0.05$) and this is within the range of chance effects. Analyses of pairwise *t*-test comparisons¹⁴ of *Adh^F* frequencies of each area within a period show no significant difference between areas. However, significant differences are observed for pairwise analyses of strain tolerances (Table 2).

The cellar section of the population is significantly more tolerant than other sections of the population at all times. This agrees with previous results⁹. However, there are also obvious distributions of tolerance phenotypes within the outside section of the population that are dependent on the collection period. During vintage the level of tolerance increases with proximity to the cellar, but this clinal distribution is transient, as during non-vintage periods the distribution of tolerance phenotypes outside the cellar is relatively uniform (Tables 1 and 2). These observations can be associated with the distribution of alcohol fumes as the fumes apparent in the fermentation area at vintage would become less concentrated as the distance from this area increased. The presence of alcohol in the outside environment in non-vintage periods would be greatly reduced. The distribution of tolerance phenotypes is apparently determined by selection in the alcohol gradient. However, this cannot be directly related to changes in *Adh* frequencies. If tolerance was mainly determined by this locus

Table 1 Ethanol tolerance and *Adh^F* frequency from population samples during vintage and non-vintage periods

Collection site	Distance from cellar (m)	Vintage				Non-vintage			
		Ethanol tolerance (no. of strains)	($\bar{x}\%$)	<i>Adh^F</i> frequency (no. of individuals)	($\bar{x}$)	Ethanol tolerance (no. of strains)	($\bar{x}\%$)	<i>Adh^F</i> frequency (no. of individuals)	($\bar{x}$)
Cellar	0	60	38.8	584	0.68	60	35.9	814	0.67
Fermentation area	3 (vertical)	60	32.6	588	0.71	40	27.5	105	0.72
1	4–7	60	28.6	561	0.67	48	22.3	345	0.70
2	13–23	60	22.0	199	0.71	40	22.9	76	0.72
3	33–63	60	20.4	204	0.66	40	20.2	50	0.67

Table 2 Pairwise *t*-test comparisons of ethanol tolerance and *Adh*^F frequency at vintage and non-vintage periods

Cellar	Fermentation area				Non-vintage
	Cellar	1	2	3	
Fermentation area	3.24†	5.04‡	4.21‡	5.04‡	
1	1.12	1.01	0.90	0.00	
2	4.92‡	1.71	1.38	2.17*	
3	0.36	0.39	0.00	0.63	
	2.12*	0.18	0.35	0.62	
	1.06	0.43		0.73	
	8.13‡	3.36†		0.60	
	0.79	1.05			
	8.77‡	4.11‡	0.79		
	0.52	0.26	1.08		
	Vintage				

**P* < 0.05, †*P* < 0.01, ‡*P* < 0.001

Analyses after angular transformation of the original data.

Upper figures are for ethanol tolerance, lower figures are for *Adh*^F frequency.

a relationship between tolerance and *Adh* allele frequencies should be apparent³. This is not the case.

Alcohol tolerance in the Chateau Tahbilk population is polygenically determined⁹. Relatively major genetic effects have been localised to chromosome II (in the vicinity of the *dp* locus which is approximately 37cM from the *Adh* locus¹⁵) and chromosome III (in the vicinity of the *e* locus) (unpublished data). At Tahbilk the response strategy of the population to alcohol fumes is an increase in the frequency of 'tolerant' alleles at loci largely independent genetically of the *Adh* locus. However, these loci and others may be functionally related to *Adh*^{16,17}. Specific activity of an enzyme of an *Adh* genotype may be influenced by modifiers¹⁷⁻²¹ which may obscure the relationship between the *Adh* locus and the selective force. Perhaps tolerant genotypes are more effective at restricting ethanol entry to cellular *Adh*. Though less likely, these results could also be interpreted as a behavioural response by which the more alcohol-sensitive genotypes differentially move away from the alcohol source. Behavioural responses of this sort have been recorded in the development of insecticide resistance²². In any event the genotypic response to environmental alcohol would seem, from our data, to be independent of the *Adh* locus.

The levels of stress caused by the concentrations and variety of alcohols encountered in different fermentation residues may influence the adaptive response of a particular population. When the alcohol stress exceeds the level at which it can be accommodated by the primary adaptive system for alcohol tolerance described here, selection may act directly on the *Adh* locus¹⁸. That is, *Adh* frequency changes will occur and alcohol resistance will evolve. Under these circumstances it seems reasonable that the mechanism of adaptation of *D. melanogaster* will not be the same in all alcohol-associated environments. For example, this is certainly so in the evolution of insecticide tolerance and resistance²³⁻²⁶.

It should be emphasised that factors other than the profile of alcohols in a substrate may also affect the *Adh* locus. The thermal stabilities of the enzymes⁴ can be correlated with allelic frequencies of natural populations and with the ability to withstand temperature shock²⁷⁻³⁰. Moreover, frequency-dependent selection may also be important at the locus^{31,32}. The relative importance of each factor and the interaction between them may therefore vary from population to population.

The *Adh* polymorphism at 'Chateau Tahbilk' is probably selectively maintained as similar genotypic frequencies have been observed in the cellar population over a number of years, despite considerable potential for random influences during annual population bottlenecks¹³. The maintenance cannot be explained by ethanol resistance alone. Only a detailed temporal study of the population biology will enable the mechanisms of maintenance of the polymorphism to be elucidated¹. Such work is currently in progress at Tahbilk.

We thank Professor A. Robertson for comments on the

manuscript and Jane Freedman, Liz Mintzis and Leanne Ritchie for assistance. The research was partly supported by ARGC grant D2-75/15072.

JOHN A. MCKENZIE

Department of Genetics,
The University of Melbourne,
Parkville, Victoria 3052

STEPHEN W. MCKECHNIE

Department of Genetics,
Monash University,
Clayton, Victoria 3168, Australia

Received 20 October 1977; accepted 13 January 1978.

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Protein polymorphism and the rate of loss of duplicate gene expression

THE adaptive significance of the widespread protein polymorphism found in natural populations continues to be a central issue in population genetics¹⁻³. Whether this variation is maintained by some form of balancing selection (the selectionist hypothesis) or by the stochastic interaction between selectively neutral mutations and genetic drift (the neutralist hypothesis) remains unclear. Also, evidence is accumulating that the simple electrophoretic separation of proteins detects a much smaller amount of the actual genetic variation present than had been assumed⁴⁻⁹. This has serious implications for much of the data used as evidence in the selection-neutrality controversy. If electrophoretic allozymes are actually large heterogeneous classes of alleles (that is, electromorphs¹⁰), then much of the most convincing data presented as evidence for the selectionist hypothesis may be illusory⁹. Therefore, methods are needed to distinguish between the selectionist and neutralist models without being dependent on detecting all or most of the genetic variation at a particular locus. I describe here a method meeting this criterion by using the relative rate of loss of duplicate gene expression to test the predictions of these two conflicting hypotheses. I report that the rate of loss for different proteins following tetraploidy in two independent groups of fish is positively correlated with the tendency for particular proteins to be polymorphic in natural populations of animals. This relationship is predicted by the 'neutralist' hypothesis of protein polymorphism and is in direct conflict with the predictions of the 'selectionist' hypothesis.

Many groups of organisms have undergone extensive gene duplication. The existence of polyploids is well known in the plant kingdom, but it is only recently that the importance of

polyploidy in vertebrate evolution has been recognised¹¹⁻¹⁴. Ohno has proposed that at least five families of fish have separately gone through a tetraploid event at some recent point in their evolutionary history¹⁵. Instances of much more recent polyploid events have been reported in several amphibian species¹⁶.

What is the expected fate of this extensive gene duplication which accompanies polyploidy? It has been proposed that many of the duplicate gene copies will become silenced through the fixation of non-functional (null) mutations¹⁷⁻¹⁹. This expectation is based on a simple mathematical model¹⁹. A null mutation occurring at one of the duplicate loci would not reduce fitness and so would behave as a neutral mutation, as long as the other locus produced enough gene product to maintain normal function. Such a neutral mutation will become fixed in a diploid population, with a probability of $1/2N$, where N is the population size. The total number of mutations occurring in a population is $2Nu$, where u is the frequency of mutations per locus. Therefore, the rate of fixation of non-functional alleles per locus is $(2Nu/2N)$, or simply u . In the case of tetraploidy, where there are many duplications in the genome, a large number of duplicated loci are expected to become non-functional under this model.

Evidence of large-scale gene loss following tetraploidy has been reported in both salmonid and catostomid fish. Species in both of these families have lost approximately 50% of the gene duplication produced by tetraploidy^{20,21}. Both of these groups, therefore, present an excellent opportunity to examine genome evolution following tetraploidy. Not all models, however, predict the loss of duplicate genes following tetraploidy. Spofford²² examined this problem from a different perspective by questioning whether a duplication at a single locus can become established in a population through the initial occurrence of gene duplication in a heterozygote and selective advantage for the heterozygote (overdominance). She demonstrated theoretically that single locus overdominance is sufficient for the incorporation of such newly arriving duplications into a species gene pool. Her results can be directly extended to polyploidy, where the gene duplication is initially present in the population. The existence of heterozygote advantage will act to maintain the gene duplication in a state of 'fixed heterozygosity', that is, different alleles fixed at the two loci. This fixed heterozygosity acts to eliminate the segregational genetic load which accompanies a single locus polymorphism due to overdominance. My own work with the maintenance of genetic variation at duplicate loci agrees with the prediction of retention of gene duplication through the establishment of fixed heterozygosity in the overdominant case²³.

We now have two very different expected fates for a duplicated locus. The first model predicts the loss of gene duplication because of fixation of null mutations at one of the duplicated loci. The model assuming selective overdominance, however, predicts the retention of gene duplication. These opposite predictions provide a potential test for the selective and neutral models of protein variation.

There are sufficient experimental data to show that different enzymes tend to have different amounts of genetic variation in natural populations²⁴. These differences are apparently related to the intrinsic properties of the enzymes themselves, as the relative degree of variability persists across taxonomic boundaries. The validity of these observations in the present context does not depend on the detection of all existing genetic variation. Rather, it rests on the assumption that electrophoretic variation is a reliable reflection of the relative amount of genetic variation present.

Why do certain proteins tend to be more variable than others? The two conflicting models of protein variation provide different explanations. The selectionist explanation is that those proteins which tend to be polymorphic are those most often under some form of balancing selection (for example, overdominance) related to the physiological function of the protein^{25,26}. The neutralist model explains such differences by

proposing different effective neutral mutation rates for different proteins. Thus, certain proteins, because of their size, structure or function, have a larger class of possible mutations which are neutral or nearly-neutral. Under a neutral model, the amount of variation at a locus is determined by this effective neutral mutation rate and the effective population size²⁷. Therefore, those proteins with higher neutral mutation rates tend to be more polymorphic.

These two explanations can be combined with the previous predictions of the expected fate of duplicated loci to make some very specific predictions. Those proteins which tend to be polymorphic should retain gene duplication if they are variable because of their frequent association with some form of balancing selection. But if these proteins are polymorphic because of a higher effective neutral mutation rate, then they should tend to lose gene duplication. These opposite predictions of the expected relationship between the amount of polymorphism and the tendency to retain gene duplication can be tested with data available in both salmonids and catostomids^{20,21}.

My own relevant work has dealt with the formal and population genetics of biochemical genetic variation in salmonid fish of the genus *Salmo* and the closely related genus *Oncorhynchus*^{20,28,29}. The high degree of gene duplication in these species often confounds the genetic interpretation of zymograms. For example, how many loci code for an enzyme represented by a single electrophoretic band? It is generally acceptable to assume the existence of a single locus in such a case. There are, however, many enzymes in salmonids which are coded for by two loci with common alleles of identical electrophoretic mobility^{20,30}. Therefore, the number of loci coding for a single isozyme cannot be known without examining the zymogram patterns associated with allelic variation²⁰.

For these reasons, work has been done to detect allelic variation and clarify the genetic control of particular proteins in salmonids through experimental matings^{20,28}, and has resulted in detailed knowledge of the genetic control of many proteins in both *Salmo* and *Oncorhynchus*. These data can be applied to the question of the relative rate of gene loss.

It has been reported that twelve proteins in the rainbow trout (*S. gairdneri*) have either lost or retained the gene duplication resulting from tetraploidy²⁰. This classification is shown in Table 1, with the overall average heterozygosity ($\bar{H}$) for each protein. Heterozygosity values represent the average proportion of individuals heterozygous for a particular protein in natural populations. The values used are from a review by Powell²⁴ of protein variation in populations of animals and are the average values for all taxa described in that review. The only exception is transferrin, which was not reported by Powell; the value used is from an earlier review by Selander and Johnson³¹.

Table 1 Average heterozygosities of 12 proteins which have lost or retained duplicate gene expression in the rainbow trout

Retaining duplicate gene expression	Average heterozygosity
Aspartate aminotransferase	0.057
α -glycerophosphate dehydrogenase	0.039
Isocitrate dehydrogenase	0.082
Lactate dehydrogenase	0.102
Malate dehydrogenase	0.064
Sorbitol dehydrogenase	0.018
Mean	0.060
Losing duplicate gene expression	Average heterozygosity
Alcohol dehydrogenase	0.140
Esterase	0.277
Malic enzyme	0.131
6-phosphogluconate dehydrogenase	0.083
Phosphoglucomutase	0.170
Transferrin	0.103
Mean	0.151

The average heterozygosity of proteins losing gene duplication in the rainbow trout is approximately 2.5 times that of proteins retaining gene duplication. This difference is exaggerated by the exceptionally high heterozygosity of esterase, but even excluding esterase in the comparison, the average heterozygosity of proteins not retaining gene duplication ($H = 0.125$) is twice that of those retaining it.

These results with rainbow trout are in agreement with predictions of the neutral model of protein variation. However, they are restricted to a limited number of proteins in one phylogenetic line. Fortunately, sufficient data are available to test this hypothesis with an independent group of organisms, the catostomids. Ferris and Whitt²¹ examined the loss and retention of gene duplication following tetraploidy for 14 enzymes in 26 species representing 10 genera of catostomids. They present which enzymes have lost or retained gene duplication in each species. I have calculated the average proportion of gene loss for each enzyme from their data. For example, 22 of the 26 species have retained duplicate gene expression for 6-phosphogluconate dehydrogenase; this represents a 15% loss of gene duplication.

The average proportion of gene loss versus average heterozygosity in all taxa (data from Powell) for each enzyme is shown in Fig. 1. Of the 14 enzymes used by Ferris and Whitt, only creatine kinase and superoxide dismutase were excluded from this analysis as heterozygosity values for these proteins are not given by either Powell or Selander and Johnson. These results also show a strong association between the tendency to lose duplicate gene expression and a high degree of polymorphism in natural populations (Spearman rank coefficient = 0.727; $P < 0.01$). This and the data from the rainbow trout are strong evidence for the neutral model of protein variation, and are in direct conflict with the predictions of the selection model.

Another way of examining the data presented here might be to compare the tendency to lose or retain gene duplication versus actual heterozygosities within that taxon (that is, the rainbow trout or catostomids). The value of such a comparison, however, is severely limited for several reasons. The first problem is measuring heterozygosities for a two-locus system at which loci share identical alleles. In this case, one electrophoretic phenotype can represent several different genotypes²⁰, making it impossible to distinguish between heterozygotes and homozygotes with regard to the individual loci. Second, the rainbow trout is a single species; the tendency of particular proteins to be polymorphic is much better estimated by the examination of heterozygosities over many species. In addition,

the data are not available for such a comparison within the catostomids because of the extremely limited sample sizes for many species.

The evidence presented here in favour of the neutral model is of special importance for several reasons. First, these results are not dependent on the detection of genetic variation present at individual loci, but only on the loss or retention of gene expression. Second, directly opposed predictions of the neutral and selection models are tested with two separate taxa. Results with both groups are in agreement with the neutral model predictions, and therefore provide strong evidence for this model. The analysis used in this paper can also be extended to other taxa possessing extensive gene duplication.

I thank C. Daugherty and J. Felsenstein for their comments, F. Utter for his encouragement, and S. Ferris and G. Whitt for providing a preliminary copy of their manuscript, and the Danish Science Council for their support during my stay at the Department of Ecology and Genetics, University of Aarhus, Aarhus, Denmark.

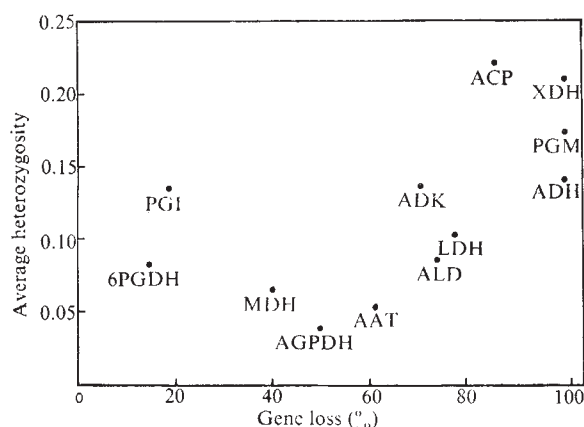
FRED W. ALLENDORF

Department of Zoology,
University of Montana,
Missoula, Montana 59812

Received 22 August; accepted 19 December 1977.

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Fig. 1 Plot of overall average heterozygosity in natural populations of animals versus proportion of gene loss in 26 species of catostomid fish for 12 enzymes. Abbreviations: AAT, aspartate aminotransferase; ADH, alcohol dehydrogenase; ADK, adenylate kinase; AGPDH, α -glycerophosphate dehydrogenase; ALD, aldolase; AP, acid phosphatase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; XDH, xanthine dehydrogenase.



High frequency recombination in centromeric and histone regions of *Drosophila* genomes

DNA SYNTHESIS in eukaryotes is asynchronous, with heterochromatin replicating later than euchromatin¹. In *Drosophila*, autoradiographic studies of metaphase chromosomes from embryonic² and cultured³ cells have shown that the centromeric heterochromatin of chromosome 2 is late replicating. The histone genes have been localised to subsections 39D and 39E in the left arm chromosome 2, close to the centromere⁴, and may be part of this late-replicating region for the following reasons. First, the five tandemly arranged histone coding sequences are reiterated approximately 50 times, and late-replicating DNA has been shown to contain significantly more repeated (but non-satellite) sequences than the genome as a whole⁵. Second, the histone genes are transcriptionally active in all but the final hours of the S phase, suggesting that their replication may be delayed⁶. Third, they are in close proximity to the centromere. Asynchrony has been detected in the

recombination process as well. Heat treatment of *Drosophila* oocytes at sequential intervals during premeiotic-S phase elicits increases in recombination that follow a well-ordered pattern⁷. To investigate the possibility that the pattern of heat response parallels that of replication, the centromeric heterochromatin of chromosome 2 and the contiguous histone region have been examined for the time of their recombinational response to heat during the S phase. We present here evidence for late response in both regions. In addition, a level of response roughly two orders of magnitude greater than the average encountered in the genome has been found in these regions.

A well-synchronised oocyte sample may be obtained for genetic and cytological studies by using the pupal system⁸. This method restricts egg recovery to a sample representing $\frac{1}{3}$ to $\frac{1}{2}$ of the first egg set. (A set is defined as 30–40 eggs, one from each of the 30–40 ovarioles making up the two ovaries of a female.) In females developing at 25 °C, the first oocyte is formed in each ovariole between 126 and 132 h (refs 9, 10), corresponding closely to pupation time, which occurs at 132 h. Premeiotic-S rapidly follows oocyte formation and occupies approximately 30 h, that is, 132–162 h (ref. 7).

To determine the time during the S phase at which the two regions respond to heat, treatments of 12 h at 35 °C were given to different groups of properly marked females, beginning at 120 h and terminating at 168 h. In all, six treated groups and one control group were studied. The histone region was demarcated by two closely flanking markers, *Bristle* (2L-54.8) to the left and *light* (2L-55.0) to the right; the contiguous centromere region was demarcated by *light* to the left and *straw* (2R-55.1) to the right. The locus of *light* lies within the centromeric heterochromatin of chromosome 2, and the locus of *straw* lies at its right boundary¹¹.

The effect of heat on recombination in the two regions is shown in Table 1. Recombination frequencies in the control (no heat) are 0.00042 for the histone region and 0.00047 for the centromeric region. At the time of peak response the values for the two regions are 0.0134 and 0.0169, corresponding to a 3,200% and 3,600% increase, respectively. A comparison of the magnitude of increase for the two regions with that observed for the total genome during the same time interval⁷ is shown in Fig. 1. A peak increase of 40% for the total genome is approximately two orders of magnitude less than those found in the histone and centromeric regions.

The total genome peaks with a treatment given between 138 and 150 h, that is, during the first 60% of the S phase. Peak responses for the histone region and the centromere region occur 18 h and 12 h later, respectively—during the last 20% and 40% of the S phase. Following these peaks, recombination drops precipitously in both regions. Failure to attain control levels by the end of the S phase is attributable to a small degree of asynchrony in the samples.

Previous studies have shown that the sensitive period for heat-enhancement of recombination throughout the *Drosophila* genome^{7,8} and heat-induction of recombination in chromo-

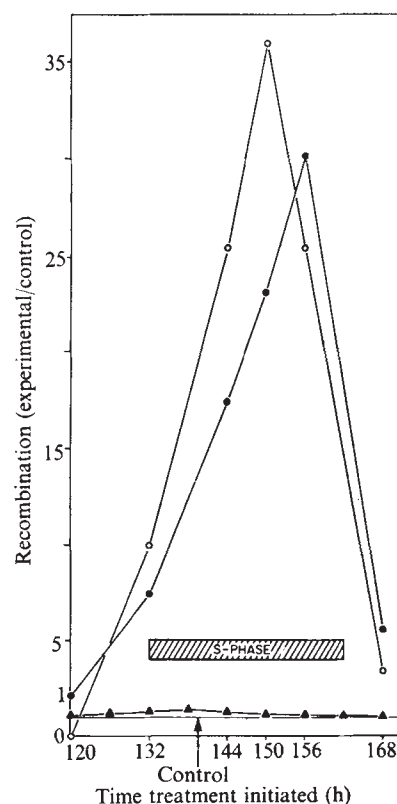


Fig. 1 Time of recombination response to heat treatment in the histone region and centromeric heterochromatin of chromosome 2 as compared with the time of response of the total genome and the time of premeiotic S. Each point on the graph represents the initiation time of treatment which extends for 12 h at 35 °C. ●, Histone region; ○, centromeric heterochromatin of chromosome 2; ▲, total genome.

some 4 (ref. 12) coincides with premeiotic-S. Electron micrographs and electron micrographic autoradiographs have demonstrated that synaptonemal complexes are present throughout the S phase in *Drosophila melanogaster*^{7,13}, and a similar situation has been reported for wheat¹⁴. Thus in these organisms, at least, homologues are synapsed and in suitable proximity for exchange during premeiotic-S. The evidence presented here suggests a close temporal correlation between replication and recombination in specific regions. Late replication in the centromeric heterochromatin of chromosome 2 is correlated with a dramatic increase in recombination, induced by a heat treatment during the last two-fifths of the S period. The response in the histone region occurs still later, during the last fifth of the S phase, and the evidence for late histone replication has been noted above. The magnitude of response in both regions may be related to the amount of AT-rich DNA sequences present. A DNA satellite which denatures 9 °–20 °C lower than the other three major satellites is found in the centromeric heterochromatin of chromosome 2 (refs 15, 16); the five structural histone genes are separated from one another by AT-rich spacers¹⁷.

This work was supported by the Department of Energy under contract with the Union Carbide Corporation.

Note added in proof: Melli *et al.*¹⁸ find synthesis of histone messenger occurs throughout the cell cycle but a detailed study of its synthesis during S has not been reported. Cessation of transcription of the histone genes during their replication remains a possibility.

R. F. GRELL

Biology Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

Received 11 November 1977; accepted 3 January 1978.

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Table 1 Recombination in the histone and centromeric regions of chromosome 2 in response to heat treatment (35 °C, 12 h) during the S phase

Treatment time (h)	No.	Recombination in the histone region	E/C*	Recombination in the centromere region	E/C*	Doubles
Control	16,866	0.00042	—	0.00047	—	0
120–132	2,200	0.0009	2.1	0.0	0.0	0
132–144	4,229	0.0031	7.4	0.0047	10.0	1
144–156	4,130	0.0073	17.4	0.0119	25.3	2
150–162	7,141	0.0097	23.1	0.0169	36.0	2
156–168	14,814	0.0134	31.9	0.0119	25.3	6
168–180	6,429	0.0023	5.5	0.0016	3.4	0

*E/C = experimental/control.

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Unequal crossing over at the rRNA locus as a source of quantitative genetic variation

MUTATIONS, in the broad sense, are the source of genetic variation for evolutionary change, whether through the agency of natural or artificial selection. The contributions of new mutations to genetic variation in artificial selection lines are thought to be small, as estimates of the rate of generation of new genetic variation by mutation have been only of the order of 10^{-3} times the environmental variance for the character per generation¹. However, several occurrences in selection lines^{2,3} have led us to question this view. We set out to measure the contribution of 'mutations' (from whatever source) to selection response for a quantitative character by selecting in lines from a largely homozygous base stock of *Drosophila melanogaster*. In two of the low lines response due to *bobbed* alleles was detected. This has allowed us to implicate unequal crossing over as a source of quantitative genetic variation and to question current views concerning the rate at which new genetic variation arises.

The base stock was constructed by methods given in ref. 4. It was homozygous for X-linked genes, had a low residual level of autosomal genetic variation and was marked with *sparkling*^{poliort} as a check against contamination. Three high (HA, HB, HC) and three low (LA, LB, LC) abdominal bristle selection lines plus 12 control lines (CA-CL) were founded from this base stock. All lines had 15 pairs of parents per generation for the first five generations and 10 pairs thereafter. Selection was at an intensity of 33½%.

The notable features of the response to selection (Fig. 1a and b) were the sudden rapid bursts of selection response in females but not males in lines LA and LC between generations 33-37 and 18-21, respectively. There were other corresponding sex-limited changes in these two low lines, namely an increase in phenotypic variance in females, an increase followed by a decrease in heritability in females, the appearance of a mutant phenotype in females but not in males, a change in the sex ratio among emergences to a deficiency of females and later emergences of females. All these changes were shown to be due to the fixation of *bb* alleles in the X chromosomes (but not the Y chromosomes) of these two lines. The sex-ratio abnormality was shown to be due to the X chromosome, to be recessive, and to be allelic in LA and LC. The sex-limited selection response was allelic in LA and LC and was shown to be due to the X chromosomes by substituting X chromosomes from all selection lines plus three control lines into a common autosomal genetic background (Table 1). The increased phenotypic variance was also attributable to the X chromosome. The X chromosomes of LA and LC were shown by test crossing to known *bobbed* stocks to be homozygous for *bobbed* alleles, whilst the Y chromosomes were free of *bobbed* alleles. All other selection lines plus 9 out of 10 surviving controls were free of X chromosome *bobbed* alleles (one control line had a moderate frequency of a much milder *bobbed* allele). Deletion mapping has located the sex-limited effects on bristle number in LA and LC at the *bobbed* locus.

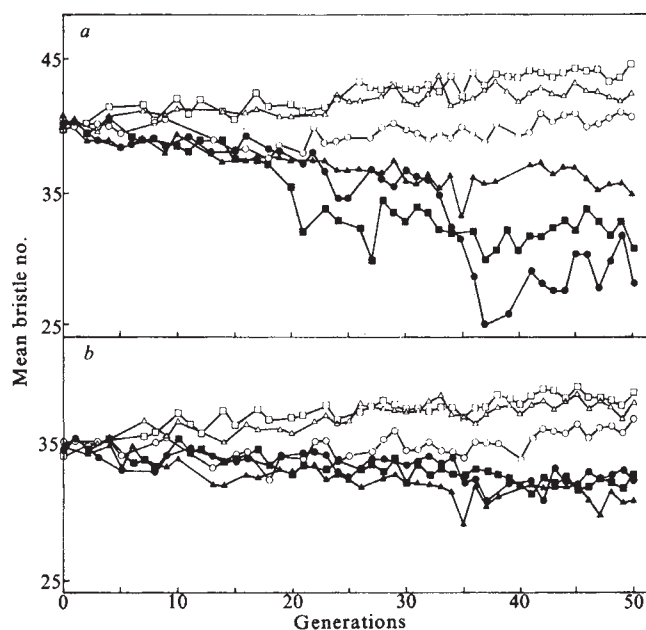


Fig. 1 Response to selection for high and low abdominal bristle number in females (a) and males (b) in the selection lines. The selection response is largely sex-limited in lines LA and LC. The figure is plotted on the scale of logarithm of one plus bristle number for reasons given elsewhere⁴. ○, HA; △, HB; □, HC; ●, LA; ▲, LB; ■, LC.

The *bobbed* locus is known^{5,6} to code for 18S and 28S ribosomal RNA, to have a multiple copy, tandemly repeated structure and to be located on both the X and Y chromosomes in *D. melanogaster*. *bb* alleles are known^{5,6} to be partial deficiencies of this locus. In addition, Schalet⁷ has shown that unequal crossing over at this locus generates new alleles. We argue that the *bb* alleles in our LA and LC lines arose by unequal crossing over. This documents a new source of quantitative genetic variation.

Similar patterns of sex-limited selection response were observed by Clayton and Robertson⁸ in all five of their low abdominal bristle selection lines and there is circumstantial evidence to implicate *bb* alleles. A. Robertson has subsequently proved *bb* to be present in a line exhibiting similar behaviour and derived from the same base population (personal communication).

The more general significance of our results is that in all eukaryotes so far studied the rRNA loci have multiple copy tandemly repeated structures at which similar events to those we have documented could occur. Certainly, genetic variation in the number of copies of the rRNA locus has been documented in several species⁹⁻¹⁴. The functioning of this locus is crucial to the expression of all protein specifying loci and thus

Table 1 Mean bristle numbers of stocks with X chromosomes from the selection lines or controls and common autosomal genetic backgrounds

Source of X chromosome	♀	♂
HA	1.613* ± 0.019	1.552 ± 0.019
HB	1.626 ± 0.019	1.557 ± 0.019
HC	1.630 ± 0.019	1.562 ± 0.019
LA	1.542 ± 0.038	1.545 ± 0.019
LB	1.633 ± 0.019	1.558 ± 0.019
LC	1.541 ± 0.042	1.542 ± 0.019
CD	1.622 ± 0.019	1.561 ± 0.019
CE	1.625 ± 0.019	1.551 ± 0.019
CF	1.636 ± 0.019	1.566 ± 0.019

Values are means ± s.e.m.

*Scale transformed to logarithm of one plus bristle number (see ref. 4).

its status is likely to influence a wide variety of characters in any organism. Unequal crossing over is clearly expected to generate genetic variation at other multiple copy, tandemly repeated loci.

A most important implication of our results is that the rate at which genetic variation is generated may be considerably higher than currently thought. Estimates of the rate of generation of new genetic variation by unequal crossing over at the rRNA locus in *D. melanogaster* are much higher than conventional mutation rates⁷. Clearly, the questions of the mechanisms and rates of origin of new genetic variation require careful re-examination.

This work was supported by Australian Research Grants Commission and Macquarie University research grants. Part of this work was done at the University of Edinburgh. We thank Miss A. Gorry and Mrs A. Hatley for technical assistance.

R. FRANKHAM
D. A. BRISCOE
R. K. NURTHEN

School of Biological Sciences,
Macquarie University,
North Ryde, New South Wales 2113,
Australia

Received 14 November; accepted 21 December 1977.

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Separate mRNAs code for tubulin subunits

MICROTUBULES are a common feature in eukaryotic cells. They have been implicated in several functions including neurite growth, axoplasmic flow, cell division and excretion¹. They are polymers of a 110,000 molecular weight heterodimer, tubulin^{2,3}. The subunits, α and β tubulin, seem to have the same molecular weight, 55,000, but their amino acid compositions, although similar, are not identical^{3,4}. Furthermore, the first 25 residues at the N terminus have been sequenced, and the α and β chains differ at 14 (ref 4). α Tubulin has been resolved into two components by hydroxylapatite chromatography^{5,6} and electrophoresis in polyacrylamide gels containing SDS and urea⁷. Mitotic microtubules and ciliary doublet A tubules from the sea urchin contain both forms of the α subunit, but flagellar doublet microtubules from the same organism contain only one form⁷. The α forms seem to differ in charge rather than size. In view of this heterogeneity of the tubulin subunits, it is reasonable to expect that eukaryotes have more than one tubulin gene. Here we present evidence that there are at least two tubulin genes. We have isolated two tubulin mRNA fractions from chick embryo brain. One codes for two α subunits, and the other codes for the β subunit.

Polysomes were isolated from the brains of 13-d chick embryos on discontinuous sucrose gradients using heparin to inhibit RNase in the homogenate. Poly(A)⁺ RNA was separated from the polysomes by affinity chromatography on oligo dT-cellulose and analysed by electrophoresis in an agarose slab gel in the presence of 6 M urea. When the gel was stained with ethidium bromide and viewed in ultraviolet light, four faint, but reproducible, bands stood out from the typical distribution of mRNA (Fig. 1A, B, C and D). To identify the RNAs in these bands we eluted them from the gel and translated them in a wheat embryo cell-free system. RNA which had been stained with ethidium bromide had very low template activity, even though we attempted to

remove the dye with phenol extraction and affinity chromatography on oligo dT-cellulose. Therefore, we extracted RNA from the gels using stained parallel lanes as markers. After translation the products, which were labelled with ³⁵S-methionine, were analysed by two-dimensional gel electrophoresis followed by autoradiography. α_1 (the slower moving form of α), α_2 and β tubulin accounted for 10% of the total incorporation when poly(A)⁺ RNA directed the wheat embryo system (Fig. 2a). α_1 and α_2 appeared in approximately equal amounts. Band A RNA directed the synthesis of mainly α_1 and α_2 tubulin, again in approximately equal amounts (Fig. 2b), and band B RNA directed the synthesis of mainly β tubulin (Fig. 2c). Band C RNA directed the synthesis of β and γ actin (data not shown). Although there were other mRNAs in these fractions the translation products of which can be seen in the gels, there was no cross-contamination between α mRNA and β mRNA. The two-dimensional gels of Fig. 2b and c should not be taken as representative of pure mRNA fractions. Not all proteins are soluble in the isoelectric focusing sample buffer, and some proteins may run off the focusing gel. The conclusion we wish to draw from this experiment is that two separate mRNAs code for the tubulin subunits. There are therefore at least two tubulin genes active in the developing chick brain.

It is not known whether α_1 and α_2 are the products of separate mRNAs which were isolated together in band A or the result of a post-translational modification. However, if the latter is true, then all of the machinery for the modification must exist in the wheat embryo extract.

Although α and β tubulin mRNAs could be separated by

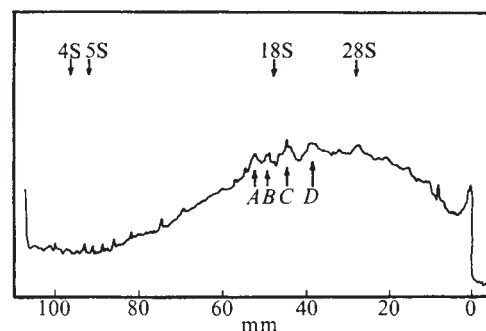


Fig. 1 Acid-urea agarose gel electrophoresis of poly(A)⁺ RNA. Freshly-dissected brains (40 g) from 13-d chick embryos were homogenised in 100 ml 25 mM Tris-HCl, pH 7.4, 3 mM Mg (OAc)₂, 40 mM KCl, 1% Triton X-100 and 0.8 mg ml⁻¹ heparin using a large Dounce homogeniser and six passes with the A pestle. All glassware and solutions had been autoclaved, and everything was kept below 4 °C after dissection. The homogenate was centrifuged 10 min at 10,000 r.p.m., and the resulting supernatant was layered over six step-gradients made with 10 ml 0.5 M sucrose over 10 ml 2.0 M sucrose. Gradients were made in Spinco polyallomer tubes for the SW27 rotor, and the buffer was the same as the homogenising buffer without Triton or heparin. Polysomes were collected as a pellet by centrifugation for 24 h at 27,000 r.p.m., washed with water, and dissolved in 6 ml water by shaking gently. Insoluble material was removed by centrifugation for 10 min at 10,000 r.p.m., and the polysomes were stored at -20 °C. Frozen polysomes were thawed in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.4 M NaCl and 0.5% SDS at room temperature and a concentration of 1 mg ml⁻¹; and poly(A)⁺ RNA was isolated by affinity chromatography on oligo dT-cellulose as described in refs 12 and 13. Poly(A)⁺ RNA (20 µg) was analysed in a 2% agarose slab gel (3 × 120 × 150 mm). The gel buffer and tray buffer, which was recirculated, contained 25 mM citric acid and 6 M urea¹⁴. The sample was dissolved in 10 µl water; 90 µl 99% formamide were added; and the RNA was denatured for 5 min at 65 °C. Just before it was layered in a 10-mm slot, the sample was coloured with 5 µl 0.1% bromophenol blue. The gel was run at 30 mA and 4 °C for 17 h, after which time the bromophenol blue had migrated 90 mm. It was stained for 30 min in 5 µg ml⁻¹ ethidium bromide and 0.5 M NH₄OAc, and photographed in ultraviolet light. The figure shows a densitometer tracing made from the photographic negative. Salient bands are marked A, B, C and D. rRNA markers from an adjacent lane are indicated.

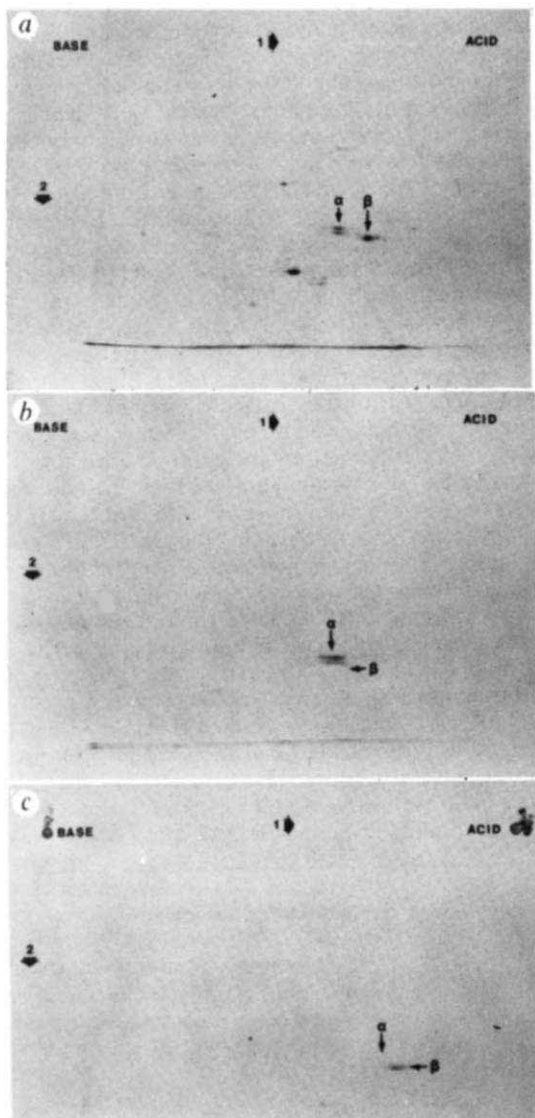


Fig. 2 Two-dimensional gel electrophoresis of *in vitro* translation products. Poly(A)⁺ RNA was fractionated in a slab gel similar to the one described in Fig. 1, but the sample, which contained 400 μ g RNA, was layered in one slot which ran almost the width of the gel. After the run, strips 10 mm wide were cut from each edge and the middle. The strips were stained with ethidium bromide as described in Fig. 1, and after the gel was re-assembled the bands A, B, C and D were cut out of the unstained sections by referring to the stained strips illuminated with ultraviolet light. The excised gel pieces were placed in Pasteur pipettes which had been prepared by removing the long tip and attaching dialysis tubing to the constricted end. The constriction prevents the gel slice from falling into the dialysis bag. The pipettes were placed in a standard tube gel electrophoresis apparatus with a tray buffer of 89 mM Tris, 2.5 mM EDTA, 89 mM boric acid and 0.1% SDS. RNA migrated into the dialysis bag overnight at a constant voltage of 120 V, and it was freed of impurities by passing it over a small oligo dT-cellulose column. The RNAs were translated in a wheat embryo cell-free system described in ref. 15, using ³⁵S-methionine to label the products. The reaction mixtures were lyophilised and analysed by two-dimensional gel electrophoresis described by O'Farrell¹⁶. Purified chick embryo brain tubulin, prepared by the method of Shelanski *et al.*¹⁷, was added to each sample as an internal marker. After electrophoresis, the gels were stained with Coomassie blue according to Fairbanks *et al.*¹⁸. When these were dry, they were marked with radioactive ink to facilitate aligning the subsequent autoradiographs with the gels. Autoradiographs were prepared on Kodak XRP-5 film. The large arrows and numbers indicate the direction of migration in the first dimension, isoelectric focusing, and in the second dimension, a 7.5% polyacrylamide gel. *a*, Products directed by poly(A)⁺ RNA (38,000 c.p.m. were layered, and the film was exposed 24 h); *b*, products directed by band A RNA (23,000 c.p.m. were layered, and the film was exposed 25 h); *c*, products directed by band B RNA (22,000 c.p.m. were layered, and the film was exposed 22 h).

electrophoresis in acid-urea agarose gels, it was doubtful that their migration in the gels was a function solely of molecular weight. RNA with secondary structure will be more condensed and will migrate faster in gel electrophoresis than an RNA of the same molecular weight but fully extended configuration⁸. Urea does not denature RNA well, and even 100% formamide will not completely denature RNA-RNA hybrids at room temperature⁹. Bailey and Davidson¹⁰ recently introduced the use of the reversible denaturing agent, CH₃HgOH, which completely destroys secondary structure in RNA by reacting with the imino NH of uridine and guanosine. We measured the molecular weights of the tubulin mRNAs by electrophoresis of poly(A)⁺ RNA in a cylindrical agarose gel in the presence of 5 mM CH₃HgOH. In another gel run in parallel, *Escherichia coli* 16S and 23S rRNAs were used as markers with molecular weights of 525,000 and 1,050,000, respectively¹¹. After the run, the positions of the markers were found by staining with ethidium bromide. The poly(A)⁺ RNA gel was soaked in dithiothreitol to remove CH₃Hg⁺. It was then sliced into 1-mm pieces, and the RNA was eluted and translated *in vitro*. The reaction with CH₃HgOH was completely reversible and had no deleterious effect on the template activity of mRNA. The translation products were analysed in a one-dimensional 10% polyacrylamide gel which did not separate α chains from β chains; and after autoradiography the majority of the tubulin mRNAs were found in three adjacent fractions with an observed molecular weight of 620,000 (Fig. 3). In the acid-urea gel, α and β mRNAs were separated by almost 4 mm, whereas in the CH₃HgOH gel the majority of tubulin mRNA was found in a region spanning only 3 mm. Thus, there did not seem to be any separation of α from β mRNA in the denaturing gel.

In acid-urea agarose gels, α and β mRNA could be separated because α mRNA migrated faster than β mRNA. But when the mRNAs were completely denatured with CH₃HgOH, α migrated with β . These data suggest that

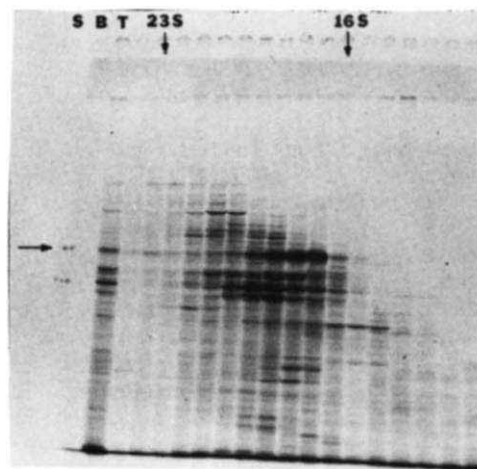


Fig. 3 Distribution of tubulin mRNA in a CH₃HgOH agarose gel. Poly(A)⁺ RNA (42 μ g) was fractionated in a 0.8% agarose gel (0.8 $\times$ 10 cm) containing 5 mM CH₃HgOH as described by Bailey and Davidson¹⁰. A parallel gel contained *E. coli* rRNA as a molecular weight marker. After 3 h at 5 mA per gel, both gels were incubated 60 min in 10 mM dithiothreitol to complex the CH₃Hg⁺. In addition, the gel with *E. coli* rRNA was stained with 1 μ g ml⁻¹ ethidium bromide. The stained gel in ultraviolet light showed that 23S rRNA migrated 36.5 mm and 16S rRNA migrated 45.2 mm. The poly(A)⁺ RNA gel was sliced into 1-mm pieces from 35 to 51 mm. The RNA was eluted from these pieces and translated as described in Fig. 2. The reaction products were analysed in a one-dimensional 10% polyacrylamide gel as described by O'Farrell¹⁶. Purified tubulin was layered in slot S. After the gel was stained with Coomassie blue and dried, radioactive ink was used to make dots over the tubulin band (arrow). Slot B contained endogenous wheat embryo reaction products (-RNA). Slot T contained total poly(A)⁺ RNA-directed proteins. Positions of 23S and 16S rRNA are indicated. Migration in the gel is from top to bottom.

α mRNA has more secondary structure than β mRNA although they have the same or similar molecular weights.

ROBERT N. BRYAN
GREGORY A. CUTTER
MASAKI HAYASHI

Department of Biology,
University of California, San Diego,
La Jolla, California 92093

Received 3 November; accepted 19 December 1977.

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Effects of cyanine dye membrane probes on cellular properties

In a number of systems, the fluorescence of a dye molecule has been useful in correlating membrane potential with biological properties of the system¹. The dye molecule can be a unique tool in cases where the insertion of microelectrodes is precluded because of interference with the biological phenomena under observation or because the cell is too small to accommodate an electrode. During studies using cyanine dyes² to correlate bacterial chemotaxis with membrane potential, side effects were observed. We report here that these side effects are caused by the interaction of light, the cyanine dye and the biological system. Although they can be avoided in many cases, the light and dye concentrations are those used in many biological studies and, therefore, the likelihood of similar perturbations in other systems must be taken into account during the design and interpretation of experiments.

Under non-stimulus conditions, *Bacillus subtilis* and *Salmonella typhimurium* display a random motility pattern in which periods of swimming in relatively straight lines (smooth swimming) are interrupted at 1–10-s intervals by abrupt changes of direction (tumbling), and this pattern is altered by attractants and repellents^{3–5}. The data in Table 1 show that the normal motility pattern is altered by visible light when the bacteria are in the presence of cyanine dyes.

The effect of light on the motility pattern of the bacteria in the presence of the dyes occurs only near the fluorescence excitation and emission wavelengths appropriate for the dye used. Thus, for 3,3'-dipropylthiodicarbocyanine iodide (diS-C₃(5)), which has excitation and emission wavelengths of 622 nm and 670 nm, respectively⁶, the alteration of motility is produced in red light at wavelengths of 630–710 nm. Similarly, two other dyes render the bacteria sensitive to blue light near the dye excitation and emission wavelengths of 470 nm and 500 nm. In each case, green light passed through a band pass filter centred at 546 nm does not affect the motility pattern.

The specific type of alteration in the swimming pattern of the bacteria depends on the intensity of the light and on the dye-to-bacteria concentration ratio, as can be seen in Table 1. High intensities of light will cause rapid paralysis of the bacteria. A lower light intensity, however, causes a brief period of constant tumbling before the onset of paralysis, and a still lower light intensity produces only constant tumbling and no paralysis. Similarly, at a given light intensity, a high concentration ratio of dye to bacteria leads to rapid paralysis, whereas at lower ratios a period of constant tumbling precedes the paralysis.

It is interesting that the light-induced tumbling is reversed when light is removed, but that the paralysis is irreversible.

The time of incubation with the dye required for the bacteria to become sensitive to visible light is different for the two species of bacteria tested. The *B. subtilis* strain is sensitive to visible light as soon as observations can be made after addition of the dye (about 15 s). The *S. typhimurium* strain, however, requires an incubation period of about 10 minutes with the dye before it becomes sensitive to visible light. These results are consistent with fluorescence measurements, which show that the dye requires a 10–15 min incubation time with *S. typhimurium* before a stable fluorescence is reached. The differing surface structures of the Gram-positive *B. subtilis* and Gram-negative *S. typhimurium* apparently affect the time required for the dye to reach its site of action at the cytoplasmic membrane.

Environmental conditions can also affect the way in which the dye-mediated light responses occur. The rapid addition of an attractant (10 mM alanine for *B. subtilis*) causes the bacteria to swim smoothly (with no tumbling) for 1–2 minutes^{4,7}. During this period of smooth swimming, the onset of the constant tumbling phase of the light response is delayed until several seconds after light is added. This is in contrast to the immediate onset of tumbling under non-attractant stimulus conditions. With *S. typhimurium* or *Escherichia coli*, in the absence of oxygen the tumbling phase of the dye-mediated light response is eliminated and the onset of paralysis is delayed.

These results are similar to those of previously published reports from our laboratory on extrinsic effects of light on bacterial motility^{8,9}, but there are some differences: (1) there is little or no period of smooth swimming between the tumbling phase and the paralysis phase in the cyanine dye-mediated response, although such a smooth phase was seen with other dyes (for example, proflavin); (2) 0.1 M L-histidine does not protect against cyanine dye-mediated light induced tumbling under conditions where 15–30 s of tumbling is seen before paralysis, although histidine does protect against a similar amount of tumbling induced in the presence of proflavine⁹. Thus, cyanine dyes may act at a site inaccessible to histidine, perhaps in the cytoplasmic membrane; (3) the cyanine dyes do not necessarily potentiate light effects which occur in the absence of added dye. Although high intensity blue light does cause constant tumbling in both *S. typhimurium*¹¹ and *B. subtilis*, an effect which was potentiated by 3,3'-dipentyl-oxocarbocyanine iodide (diO-C₅(3)) and 3,3'-dihexyloxo-carbocyanine iodide (diO-C₆(3)), no intrinsic light effect could be potentiated by diS-C₃(5) in the 630–710 nm wavelength region.

In addition to affecting swimming behaviour, the combination of cyanine dyes and visible light also affects the membrane potential and oxygen-utilising reactions of the bacteria. When the *B. subtilis* are incubated in the presence of 5 μ M diS-C₃(5) and high intensity red light (630–710 nm), there is a two- to threefold increase in the fluorescence of the dye during the first five minutes of illumination. Concomitant with this depolarisation, the bacteria are paralysed and lose the ability to take up oxygen. (The longer period in this case is caused by the decreased dye levels used in the experiment.) Changes in membrane potential are known to affect bacterial swimming behaviour^{2,10,11}, suggesting that it is the depolarisation of the membrane (perhaps due to interference with the oxygen-utilising electron transport reactions) that leads to the observed changes in the swimming behaviour of the bacteria. Manson *et al.*¹² have recently reported side effects of cyanine dyes on artificially developed proton motive force experiments which agree with this conclusion. Other dyes have been shown to mediate light-induced potential changes in neurones¹³.

The mechanisms of dye-mediated light effects and their usefulness in the study of bacterial chemotaxis and motility have been discussed elsewhere, and some similar conclusions seem to pertain to the cyanine dye mediated responses. Light can apparently induce an activated state of the cyanine dyes which can directly, or indirectly through an intermediate, modify the

Table 1 Effects of visible light on the motility of bacteria

Bacteria and environmental conditions	Dye added*	Dye concentration (μ M)	Observation wavelengths (nm)†	Light intensity‡	Motility pattern after illumination§
<i>B. subtilis</i> 2×10^7 bacteria per ml	None	—	390–710	1	Random
	DiS-C ₃ -(5)	0.5–5	390–566	1	Random
		0.5	630–710	1	Tumbling
		2.5	630–710	0.5	Random
				1	Tumbling (5–10 s) then paralysis
		5	630–710	0.25	Tumbling
				1	Rapid paralysis (no effect with 2×10^8 bacteria per ml)
				0.5	Tumbling (5–10 s) then paralysis
				0.25	Tumbling (15–45 s) then paralysis
	DiO-C ₅ -(3) or DiO-C ₆ -(3)	0.5	390–530	1	Tumbling
<i>B. subtilis</i>	None	—	526–710	1	Random
			390–530	1	Rapid paralysis
Immediately after rapid addition of 10 mM L-alanine	DiS-C ₃ -(5)	5	390–710	1	Smooth (no tumbling)
			630–710	1	Smooth (no tumbling) for 1–2 min, then random
<i>S. typhimurium</i> 2×10^7 bacteria per ml	None	—	390–710	1	Tumbling 5–10 s after illumination, then paralysis
	DiS-C ₃ -(5)	5	630–710	1	Random at first, but rapid paralysis after 10 min incubation with dye
anaerobic¶	None	—	630–710	1	Random
	DiS-C ₃ -(5)	5	630–710	1	Random, then paralysis after 30 s–1 min of illumination

Two species of bacteria were used in these experiments. *Bacillus subtilis* strain W168 and *Salmonella typhimurium* strain ST1 are both wild-type for growth and chemotaxis. The *B. subtilis* were grown on a tryptone and salts medium¹⁴ to mid-log phase and then resuspended at the indicated concentrations in the minimal salts chemotaxis medium of Ordal and Goldman¹⁰. *S. typhimurium* was grown in Vogel–Bonner salts with citrate added¹⁵, with 1% glucose as carbon source. Mid-log phase cells were collected and resuspended in the same medium for observation. Microscopic observations of the bacteria were made with a Leitz microscope and 75 W xenon light source identical with those described previously⁹, except that a dry dark-field condenser (Leitz 0.60/L11) was used in the experiments reported here.

*Dye was added to the indicated final concentration as an ethanolic solution. The ethanol concentration never exceeded 1%, a concentration which did not affect the motility or light responses of the bacteria. The dyes used and their abbreviations (see ref. 6) were 3,3'-dipentylloxycarbocyanine iodide (diO-C₅-(3)), 3,3'-dihexyloxycarbocyanine iodide (diO-C₆-(3)), and 3,3'-dipropylthiodicarbocyanine iodide (dis-C₃-(5)).

†Filters with 50% cut off at the indicated wavelengths (Baird-Atomic) were placed between the light source and the microscope stage.

‡Arbitrary units. Unfiltered light from a 75 W xenon lamp focussed through the dark-field condenser onto the bottom of a slide has an incident light intensity of about $0.2 \text{ J cm}^{-2} \text{ s}^{-1}$ (ref. 9). For a given set of cut-off filters, the incident light intensity is given the value 1. Lower incident light intensities were obtained by inserting neutral density filters.

§Unless otherwise noted, the bacteria were observed for one minute after illumination.

||Random behaviour consists of a period of smooth swimming in approximately straight lines interrupted at 1–10-s intervals by abrupt changes in direction (tumbling).

¶Bacteria were placed in air-tight chambers similar to those described previously⁹, and incubated for a time sufficient to allow utilisation of the available oxygen as measured with an oxygen electrode.

tumble frequency regulator controlling swimming behaviour. In considering the mechanism of these light responses, it is interesting that the first tumbling phase of the response is reversible when the light is switched off. This could be due either to the inherent ability of the bacteria to adapt their swimming behaviour after a stimulus^{4,5} or to the lack of a steady supply of the photoactivated species. The later stage of paralysis is probably the result of nonspecific photoinduced damage which destroys the ability of the bacteria to take up oxygen and generate the membrane potential necessary for motility; possibly a peroxide or free radical is involved.

These results show that visible light affects a basic physiological function of living bacteria in the presence of cyanine dyes, and thus that the dyes should be used with caution as probes of membrane potential. With many commonly used combinations of light source, wavelengths and slit widths, we have found that the light intensity in a fluorimeter is comparable to the light intensities that lead to the dye-mediated changes in swimming behaviour. Furthermore, dye concentrations similar to those that lead to the swimming behaviour changes are often used when the dyes are used as probes of membrane potential. The possibility of cyanine dye induced artefacts in such experiments must, therefore, be considered. These results should also be considered with others which showed that the dyes have effects on cells in the absence of light^{1,16}. Usually, dye concentrations can be manipulated either to ensure that the dye and light levels are so low that perturbations to the system are

minor or to ensure that the cyanine dye effects are understood. With the knowledge gained from such investigations, the dyes can be useful in correlating membrane potential with other biological properties.

We thank Dr A. S. Waggoner for a gift of the cyanine dyes and Dr T. J. Leighton for a gift of the *Bacillus subtilis* strain. This work was supported by a USPHS grant AM-09765 and by an NSF pre-doctoral fellowship to J.B.M.

JEFFREY BOONE MILLER
D. E. KOSHLAND JR

Department of Biochemistry,
University of California,
Berkeley, California 94720

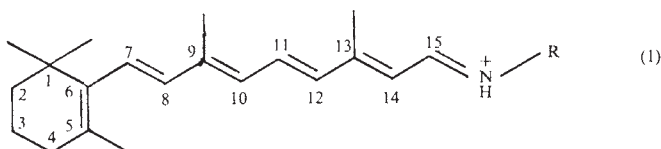
Received 2 November 1977; accepted 9 January 1978.

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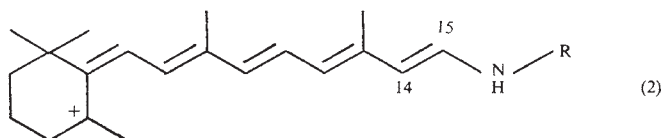
A mechanism for the light-driven proton pump of *Halobacterium halobium*

MITCHELL's hypothesis of chemiosmotic coupling between redox reactions and ATP synthesis in membranes¹ is supported by the finding of a light-driven proton pump in the purple membrane of *Halobacterium halobium*²⁻⁴. The purple membrane contains the protein bacteriorhodopsin in a crystalline array, with retinal as chromophore^{5,6}. We propose here, on the basis of quantumchemical arguments and experimental observations, that the *H. halobium* proton pump may involve proton translocation through photoisomerisation of retinal about its 14-15 single bond.

Most of the information on the light-driven proton pump of *Halobacterium* comes from spectral observations of the pump cycle⁷. Light is absorbed by bacteriorhodopsin with absorption maximum at 568 nm (B_{568}). B_{568} contains all-*trans* retinal bound as a protonated Schiff base to a lysine residue⁸⁻¹¹. *In vitro* the protonated Schiff base of retinal absorbs around 440 nm, however (ref. 12). It assumes a mesomeric structure between the polyene resonance form

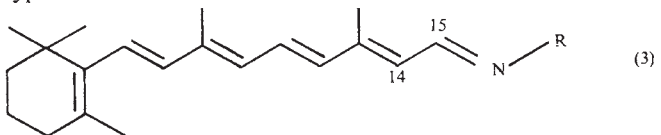


and the polyenylic ion resonance forms, for example



Stabilisation of the latter results in the bathochromic shift¹², for example to 568 nm in bacteriorhodopsin.

The longest lived intermediate of the pump cycle absorbs at 412 nm (M_{412}). In the dark M_{412} reverts to B_{568} ; a proton is released to the outer cellular space and another proton absorbed from the cytoplasmic side^{7,13}. A knowledge of the structure of the M_{412} intermediate is vital for the elucidation of the molecular mechanism of the proton pump. Lewis *et al.*^{9,10} and Aton *et al.*¹¹ concluded from resonance Raman scattering data that M_{412} represents an unprotonated Schiff base. The blue shift to 412 nm is then to be attributed to the pure polyene type structure



How do deprotonation and reprotonation of B_{568} after optical excitation induce a vectorial proton transfer against a proton gradient? Retinal is known to isomerise around its double bonds on absorption of light. As bacteriorhodopsin exhibits some steric freedom for bond rotation, proven by the conversion of B_{568} in the dark to a 50:50 mixture of all-*trans* and 13-*cis* retinal which in the light reverts to the all-*trans* chromophore¹⁴, one may expect that the chromophore isomerises during the pump cycle¹⁶ (although thermal back-isomerisation is generally slow—20 min for the *trans* $\rightarrow$ 13-*cis* isomerisation during dark adaptation¹⁴). Chemical analysis indicated that during the pump cycle bacteriorhodopsin contains solely all-*trans* retinal¹⁵, but a more recent study by Pettei *et al.*¹⁷ revealed a 13-*cis* M_{412} intermediate, a finding also supported by time-resolved resonance Raman studies¹¹. Slifkin and Caplan¹⁸ detected, by time-modulation spectroscopy, the occurrence of two different M_{412} intermediates exhibiting different polarisation behaviour. This finding has been corroborated by Hess and Kuschmiz¹⁹ and Kalisky, Lachish and Ottolenghi (in preparation). A rapid decay of light-induced linear dichroism syn-

chronous with the M_{412} intermediate indicating a conformational transition of the chromophore, has also been observed at 620 nm (ref. 20).

Thus if conformational transitions of the chromophore play a part in the pump cycle, we must explain how such an isomerisation process could induce changes of the acid-base properties of the chromophore and, thereby, a vectorial transposition of a proton.

The 14-15 single bond adjacent to the C = N group of the Schiff base of retinal exhibits some remarkable properties which makes photoisomerisation about this bond suitable for a vectorial proton translocation. According to the structures (1), (2) and (3) this bond represents a partial double bond in the protonated chromophore B_{568} but a single bond in the unprotonated intermediate M_{412} . To test this supposition we calculated potential surfaces for rotation about the corresponding 6-7 bond of the octatetraene analogue of the retinal Schiff base (Fig. 1). The protonated Schiff base indeed exhibits a high barrier for thermal isomerisation in the ground state. The unprotonated Schiff base, as expected, has a low barrier in the ground state. The energy barrier for photoisomerisation in the lowest excited state, however, is low for the protonated form and high for the unprotonated form. This behaviour of ground and excited state isomerisation potentials suggests a mechanism for the proton pump in *Halobacterium*: photoisomerisation of B_{568} around its 14-15 bond facilitated by a low energy barrier transports the nitrogen proton from an environment E_i in contact with the cytoplasmic side of the purple membrane to an environment E_f in contact with the outer cellular space. Thermal back-isomerisation is prevented for the protonated chromophore by a high barrier for thermal isomerisation, but is possible after the proton has been released at E_f because of the low isomerisation barrier of the unprotonated chromophore in the ground state. Proton diffusion between E_i and E_f without the chromophore carrier is hindered. This mechanism is supported by studies²² of *trans* $\rightarrow$ *cis* photoisomerisation of a stilbazolium betaine in the protonated form that thermally reverses only after deprotonation in a basic medium.

The 14*S-trans* $\rightarrow$ 14*S-cis* isomerisation suggested probably occurs in connection with a 13-14 double bond rotation. The steric hindrance imposed on the chromophore by the tight protein cavity of its binding site is likely to enforce an all-*trans* $\rightarrow$ 13-*cis* photoisomerisation to be accompanied by a 14*S-trans* $\rightarrow$ 14*S-cis* bond rotation (D. Oesterhelt & A. Warshel, personal communication). The reversion of the latter motion at the M_{412} stage of the cycle can be accommodated sterically by a complementary bond rotation in the lysine moiety to which the chromophore is bound.

The increase of the barrier of isomerisation on protonation implies a strong dependence of the *pK* value of the Schiff base nitrogen on the rotation about the 14-15 bond. This feature would greatly increase the efficiency of a proton pump based on the proposed mechanism. This is demonstrated in Fig. 1c which combines Fig. 1a and 1b to model the behaviour of the chromophore in bacteriorhodopsin. The potential curves in Fig. 1c corresponding to the ground and excited states of the unprotonated Schiff base R are labelled $S_0(R)$ and $S_1(R)$, respectively, representing a state $XH^+ + R$, where X represents some proton acceptor on the cytoplasmic side. The potential curves corresponding to the ground and excited state of the protonated Schiff base are labelled $S_0(RH^+)$ and $S_1(RH^+)$, respectively, representing the state $X + RH^+$. The energy difference between $X + RH^+$ and $XH^+ + R$ in the untwisted ground state has been assigned the value 0.17 eV corresponding to a difference of 3 between the *pK* values of R and X ($\Delta pK = pK(R) - pK(X) > 0$ sets $X + RH^+$ below $XH^+ + R$ by an energy $\Delta E = 2.3kT \Delta pK$). The crossing of the ground state potential curves $S_0(R)$ and $S_0(RH^+)$ implies that on bond rotation the ΔpK value reverses its sign, that is, the retinal Schiff base becomes more acid than X. Steric hindrance of the 14-15 photoisomerisation by the protein environment as indicated in Fig. 1c could fix the

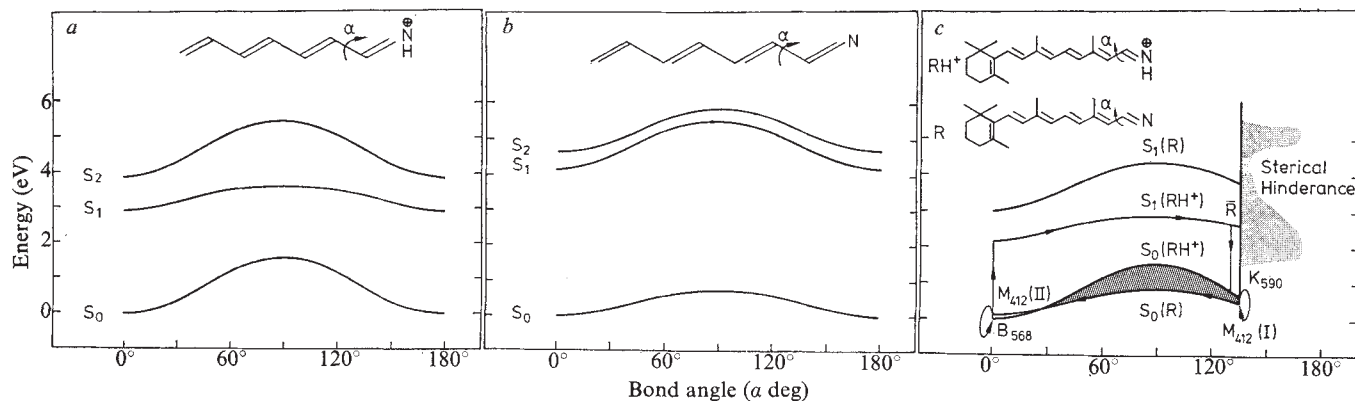


Fig. 1 Isomerisation potentials of polyene Schiff bases contributed by the π electrons and evaluated by a Pariser–Parr–Pople SCF–CI calculation including all single, double, triple and quadruple excitations from the SCF ground state; interaction parameters [ionisation energies, repulsion matrix elements (Ohno approximation) and charges] were taken from ref. 21 describing C=N like pyridine and C=NH⁺ like pyrrole; for the resonance integrals (in eV) we used the formula $\beta = -2.6 + 3.21(r - 1.397)$ where the bond distances r (in Å) were chosen to be 1.46 for single bonds and 1.35 for double bonds. *a*, Protonated Schiff base of octatetraene. *b*, Unprotonated Schiff base of octatetraene. *c*, Combination of the potentials from (*a*) and (*b*) to describe the Schiff base of retinal; the ground state energy ordering is described in the text, the excitation energies for R and RH⁺ were chosen to conform with the observed absorption maxima in bacteriorhodopsin of 412 nm and 568 nm, respectively.

chromophore in an acid conformation $\bar{R}$ thereby transforming light excitation into a change of acid–base properties. A suitable group Y near the translocated nitrogen could accept the proton as long as its pK value satisfies the condition

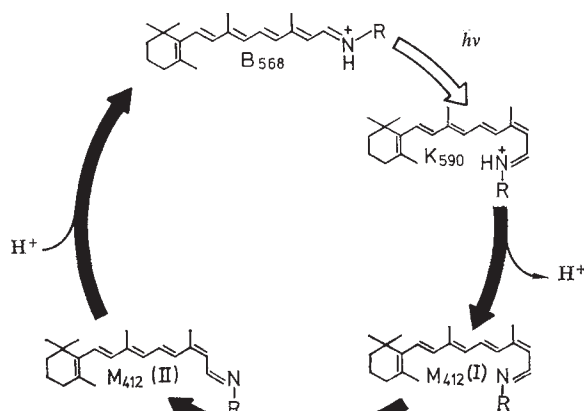
$$E(\bar{R}H^+ + X) - E(\bar{R} + XH^+) > 2.3kT[pK(X) - pK(Y)]$$

If, for example, the $S_0(\bar{R}H^+)$ state lies 0.3 eV above the $S_0(\bar{R})$ state the proton transfer occurs effectively even if the acceptor Y has a pK value 5 units below that of X. Thus, the light-driven proton pump could easily overcome a strong pH gradient.

After the sterically induced proton release described above the unprotonated chromophore has to transcend the low energy barrier in the $S_0(R)$ state. We suggest that this process (which can be accelerated by optical excitation) has been observed in the experiments described in refs 18–20 and is perhaps to be attributed with the activation energy of 13 kcal mol⁻¹ measured for the $B_{412} \rightarrow B_{568}$ dark transition²³.

The strong bathochromic shift of the Schiff base of retinal on protonation¹² reflected by the energy difference between the $S_1(R)$ and the $S_1(RH^+)$ states (Fig. 1c) implies that the light-excited chromophore is a strong base²⁴. This means that during photoisomerisation the chromophore binds the proton tightly at the terminal nitrogen. This proton in turn stabilises during photoisomerisation the chromophore binds the proton tightly at the terminal nitrogen. This proton in turn stabilises the transfer of π -electron charge from the β -ionone ring to the nitrogen in the excited state $S_1(RH^+)$ (ref. 25) and, thereby,

Fig. 2 Model of the proton pump cycle of *Halobacterium halobium*.



reduces the barrier of photoisomerisation at the $\alpha = 90^\circ$ position of $S_1(RH^+)$.

Figure 2 summarises the suggested mechanism for the light-driven proton pump of *Halobacterium* B_{568} photoisomerises about the 14–15 bond to form the first intermediate K_{590} which exists in a sterically hindered 14*S*-*cis* form. K_{590} releases (probably indirectly) a proton to the outer cellular space and goes over to the unprotonated 14*S*-*cis* M_{412} (I) intermediate. This intermediate isomerises back to all-*trans* M_{412} (II) which accepts a proton from the cytoplasmic side to form B_{568} .

We pointed out above that the 14–15 bond rotation may be induced in the first place by an all-*trans* $\rightarrow$ 13-*cis* photoisomerisation (included in Fig. 2). It is possible that thermal reversion of this double bond rotation is facilitated by reprotonation of the Schiff base in the same way as thermal rotation around the single bond is being hindered.

We thank A. Weller for encouragement and support, and the Gesellschaft für wissenschaftliche Datenverarbeitung mbH., Göttingen, for computer facilities.

KLAUS SCHULTEN
PAUL TAVAN

Max-Planck-Institut für biophysikalische Chemie,
Abteilung Spektroskopie,
D 3400 Göttingen-Nikolausberg, am Fassberg, FRG

Received 23 August; accepted 9 December 1977.

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More evidence that light isomerises the chromophore of purple membrane protein

LIGHT absorption by the purple membrane protein (bacteriorhodopsin) of *Halobacterium halobium* leads to pumping of protons across the cell membrane¹; the resulting proton gradient can in turn be used to make ATP^{2,3}. As in photosynthesis, the energy for this proton pumping must be ultimately derived from the energy of the absorbed photon which is converted into chemical free energy in the formation of the primary photochemical product, bathobacteriorhodopsin or K (refs 4 and 5) (see Fig. 1). On warming, K decays to the photocycle intermediate L and then to M⁴, which, after warming to higher temperatures, returns to purple membrane protein (PM). The chromophore of the native pigment is all-*trans* retinal attached to the protein by way of a protonated Schiff base^{6,7}. The question arises: What is the action of light on the chromophore which results in K formation? In earlier papers^{5,16}, we have provided a partial answer to the nature of K by marshalling strong evidence that, as in visual pigments⁸, the primary effect of light is to isomerise the chromophore. Pettei *et al.*⁹ have recently shown using a chromophore extraction method that by the time of M formation, at least part and perhaps all of the chromophores have been isomerised to the 13-*cis* conformation. Resonance Raman studies of the chromophore of the pigment and its M intermediate also suggest that the chromophore is isomerised from the all-*trans* to the 13-*cis* conformation by the time of M formation⁷. We show here that when M or L is irradiated at -196°C , their primary photoproducts—M' or L'—can reform the original pigment when warmed to -90°C . This property of these new photoproducts can most readily be explained if light has photoisomerised the chromophore of the intermediates back to the conformation originally present in the pigment. Thus, by the L and M intermediates in the photocycle, light has altered the geometry of the chromophore from the all-*trans* to another geometrical conformation.

The M intermediate can be formed as a pure species at -70°C in conditions of high salt concentration and high pH¹¹. At this temperature, when M absorbs a photon it reconverts to the original pigment, PM¹¹. Quite different behaviour is observed, however, when M is formed at room temperature by constant illumination with $>520\text{ nm}$ light (about 70% M, 30% PM) and rapidly cooled to -196°C (Fig. 2a, curve 1). Absorption of a photon by M at this

Fig. 1 Schematic diagram of light and dark reactions of the PM, its primary photoproduct, K, and the intermediates formed from the dark decay of K (modified from ref. 4). The intermediates are placed vertically according to their relative free energy, except the positions of L' and M', which are not yet established. Δ , Thermally activated dark reactions.

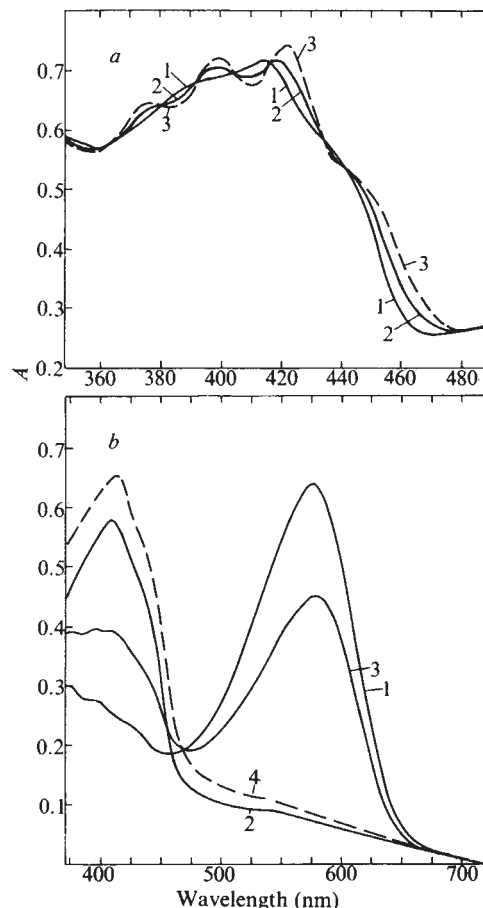
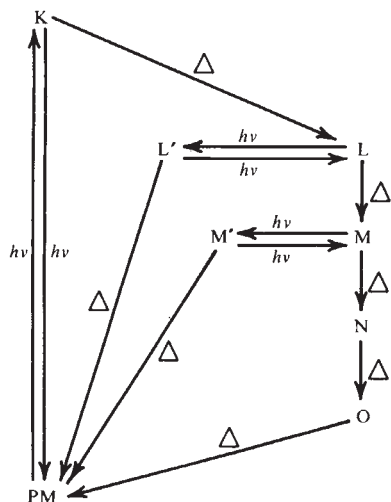


Fig. 2a, Absorption spectra at -196°C of: 1, M (about 70%) and PM (about 30%); 2, mixture of M' (about 35%), M (about 35%) and PM (about 30%) (as determined by an experiment similar to that in b) produced by 420-nm irradiation of 1 at -196°C ; 3, calculated spectrum of 70% M' and 30% PM. Small amounts of K were produced on 420-nm irradiation but we found that this did not contribute to the spectral changes shown here. Absorption spectra were recorded using a flat-windowed dewar filled with liquid nitrogen and a Cary 118 spectrophotometer. **b**, Determination of percentage M' in the 440-nm photostationary state of M and M'. Absorption spectra of: 1, 100% PM at -70°C ; 2, 100% M at -70°C ; 3, after cooling 100% M to -196°C , irradiating with 440 nm light and warming to -90°C ; 4, final conversion to M used to make corrections for scattering changes. About 63% of the M present in curve 2 was converted to PM, indicating that the photoproduct M' formed at -196°C can thermally decay to PM at -90°C . A similar experiment with 420-nm irradiation at -196°C showed that about 50% of the M present formed M' at -196°C . Spectra recorded at -90°C and -70°C were made with the dewar filled with ethanol cooled to the given temperature.

temperature does not lead to photoconversion back to PM, but rather results in the partial formation of a new product, M' (Fig. 2a, curve 2), which still absorbs at about 410 nm but shows more structure in its absorption spectrum. M' may be similar to the species observed at -10°C during the photoreversal of M by Ottolenghi *et al.*¹², and at 20°C by Hess and Kuschmitz¹³.

A similar observation^{14,15} of non-photoreversibility at low temperature has been made for the rhodopsin-bleaching intermediate, metarhodopsin I, and a similar explanation seems reasonable here: that a change in the conformation of the protein is required to return the intermediate to the original pigment, a change that cannot occur at the lower temperature. Several other observations by us¹⁶ also show that there is a change in the protein conformation in going from PM to M, so there must also be changes in the protein in going from M back to PM.

On warming the sample containing M' from -196°C to a temperature where major protein changes should more readily take place but where M cannot yet thermally revert

to PM, we have found that M' can return to PM. When 100% M at -196°C is irradiated with 420-nm light and then warmed to -90°C , we found that 63% of the original M goes on to PM (Fig. 2b). This suggests that on irradiation at -196°C , a photostationary state is formed between M and M' analogous to that produced between PM and K (ref. 4), and between rhodopsin and bathorhodopsin¹⁷.

Similarly, in Fig. 3a, we show that the L intermediate can be photoconverted to the analogous L' when irradiated at -196°C . PM is cooled to -90°C (Fig. 3b, curve 1) and irradiated with 640-nm light leading to a photosteady state containing 35% PM and 65% L (Fig. 3b, curve 2). The amount of L was determined using our own method (see ref. 16). This mixture of PM and L was then cooled to -196°C (Fig. 3a, curve 1) and irradiated with 520-nm light to form a mixture containing PM (about 24%), K (about 11%), L and the product of irradiating L at -196°C , L' (Fig. 3a, curve 2). If this mixture is irradiated with very long wavelength light (λ_{max} 640 nm), the K which was

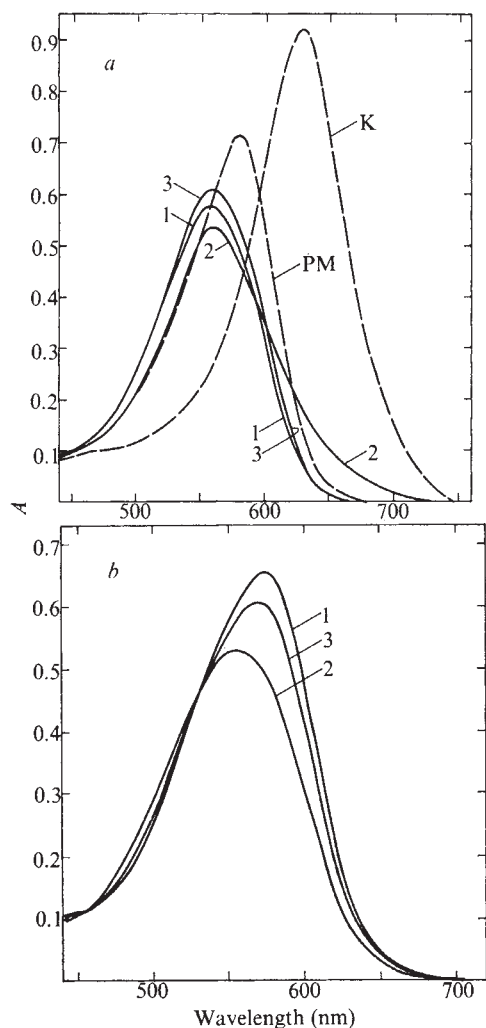


Fig. 3a. Absorption spectra at -196°C of: 1, PM (about 35%) and L (about 65%) produced by 640-nm irradiation at -90°C followed by cooling to -196°C ; 2, same mixture after 520-nm irradiation at -196°C to produce a photostationary state mixture containing PM (about 24%), K (about 11%), L (about 22%) and the -196°C photoproduct of L, L' (about 43%); 3, absorption spectrum at -196°C after the sample in curve 2 was irradiated with 640 nm light to drive K back to PM forming a mixture of only PM, L and L'. The -196°C spectra of PM and K are included for reference. **b.** Absorption spectra at -90°C of: 1, PM; 2, after irradiation with 640-nm light to form a mixture of PM (about 35%) and L (about 65%); 3, after cooling this sample to -196°C , irradiating as described in **a** to produce a mixture of only PM, L and L', and then warming again to -90°C . About 66% of the L present in curve 2 was converted to PM, indicating that the photoproduct L' formed at -196°C can thermally decay to PM at -90°C .

previously formed is returned to PM, leaving just PM, L and L' (Fig. 3a, curve 3). This figure shows that, like the M intermediate, when L is irradiated at -196°C with 520-nm light, it cannot photoconvert to PM. However, when this irradiated mixture is warmed from -196°C to -90°C , a temperature at which L cannot thermally revert to PM, much more PM is found than in unirradiated samples (Fig. 3b, curve 3), indicating that L' has reverted to PM. We found, after irradiating to form a photosteady state mixture of L and L' at -196°C and warming to -90°C , 66% of the L initially present converts to PM. This presumably represents the proportion of L' in the photosteady state mixture with L. From this calculation of the percentage of L' in Fig. 3a, curve 3, we have determined the absorption spectrum of L'. L' has an absorption spectrum which is very similar to that of its parent pigment L, but with a 3-nm red shift and a 5% increase in intensity (spectra not shown).

What does light do to the chromophore to allow the M' and L' intermediates to return to the pigment? In earlier papers^{5,17}, we argued that along with other properties, interconvertibility by light of the pigment and its primary photoproduct K suggests that the effect of light is to isomerise the chromophore. In this case, only a change in the geometry of the isomer would be necessary to change PM to K or *vice versa*. The experiments presented here can also be readily understood if light induces a change in the geometry of the chromophore of M or L back to the geometry found in PM to form M' or L'. At -196°C , the protein of M' or L' cannot refold to PM; however, the chromophore change has initiated the reformation of the pigment which can then be completed on warming to -90°C by subsequent changes in protein conformation.

An alternative hypothesis is that light forms K by initiating a photon or electron exchange with the protein¹⁰. The demonstrated photoreversibility of the intermediates K, L or M with the original pigment, however, would require that light can initiate a similar type of reverse proton or electron transfer between the protein of a photocycle intermediate and the chromophore. As such photoreactions can take place at -196°C with the protein in three different conformations (K, L and M) and the chromophore in two different states (protonated Schiff base: K, L; unprotonated Schiff base: M)^{6,7}, such a hypothesis seems very unlikely (see also refs 5 and 17).

This work was supported by NIH grants EY 01323 and RCDA EY 00025, NSF grant PCM 76-82704, NIH fellowship (HEW PHS GM 07283) to J.B.H., and Institutional National Research Service Award (HEW PHS EY 07005) to B.B.

JAMES B. HURLEY
BRIAN BECHER
THOMAS G. EBREY

Department of Physiology and Biophysics,
524 Burrill Hall, University of Illinois,
Urbana, Illinois 61801

Received 31 October 1977; accepted 3 January 1978.

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STUDENT BOOKS SUPPLEMENT

Framework for science teaching

Steven Rose

Science in a Social Context. (SISCON Series.) First six volumes. (Butterworths: London, 1977.) £1 each.

Once upon a time, science was taught either as a timeless logical construction or as an internalist chronology of discovery. For some years now the need to relocate science teaching within a framework which reveals its social, historical and epistemological bases has been apparent. Yet there has been a real dearth of available teaching materials other than those constructed by individuals to suit the particular needs of special courses. Hence the decision, taken several years ago now, to embark on a collaborative inter-university scheme, SISCON (Science in a Social Context), to produce such materials at undergraduate level, was timely. Collaborative projects between teaching institutions often sound easier in theory than they prove to be in practice; it is not just that differently perceived goals need to be melded to give a common sense of purpose, but even the sheer logistics of the organisation of team meetings or the agreement on common editorial policies may prove problematic. In a sense the SISCON series has avoided the challenge of producing a unitary—or even cross-linked—set of teaching documents and has opted for the easier approach of commissioning a number of rather loosely related short volumes (the longest of the six reviewed here is only 80 pages) which do not add up to a comprehensive course and indeed differ significantly from one another in objectives, teaching style and level of approach.

The brevity of the texts inevitably makes the treatment either introductory (in the best cases) or superficial (in the worst) and each leans rather heavily on references to additional, and sometimes essential, further reading which must, I suspect, limit their utility to institutions with well-endowed libraries. Some of the booklets themselves are largely collages of extracts from existing material. In two, the extracts form the basis of instructive suggested teaching exercises, but not all the authors have thought through, or been adequately editorially briefed on, the differences between a miniature textbook and structured educational material which can be used both by individual learners and in classes and tutorials. As a result, I would guess that the utility of these booklets as teaching texts will vary markedly.

Indeed, if I may be allowed to express a little institutional loyalty, it is both slightly surprising, and somewhat of a pity, that the Open University was not involved in the SISCON exercise, granted both the OU's expertise in the production of teaching materials and its commitment to teaching science in precisely the social context which is SISCON's concern.

Perhaps the best of the booklets reviewed here is Clive Morphet's *Galileo and Copernican Astronomy*, which uses the familiar historical episode instructively to point up differing theories of scientific knowledge and its external relations, both as perceived in the seventeenth century and in terms of the present-day Popper–Kuhn debate. An imaginative use of translations of original texts (such as Galileo's letter to the Grand Duchess Christina) give a real bite to the arguments not usually found in texts on the history of science. Morphet's text is punctuated by quite challenging self-assessment questions, although it is a pity that the utility of the booklet for the individual learner is limited as there is no feedback within the text, and the teaching relies on tutorial support that, in many institutions which one might imagine could profitably use the text, may be hard to come by.

Diana Manning's *Society and Food: The Third World*, discusses the biology, economics and politics of feeding the 'third world' using a broad-ranging selection of extracts from nutritionists, ecologists and those interested in rural development. It works hard to counterpose alternative explanations and solutions but by omitting the critique that has been developed in recent years of 'agribusiness' has missed an important element in the debate. Nonetheless, I would expect it to work well as a student text.

Research and Technology as Economic Activities (Kenneth Green and Clive Morphet) briskly develops the ideas on the relationship between research and economic growth expounded in recent years by the Sussex and Manchester schools. It is disappointingly somewhat trapped in orthodox economic theories which the science student will have to receive uncritically, and its statistical source material is generally rather out-of-date. Relative research and development expenditure, nationally and internationally, has shifted markedly since the mid-1960s and unless it is planned to update

the text frequently, will seem almost archival within a few years. However, the case studies of the research practice of actual companies give the student a little more to bite on, and some thought has gone into structuring questions and discussion points.

Leonard Isaacs offers a quite different approach in *Darwin to Double Helix: The Biological Theme in Science Fiction*, which counterposes biological developments over the past century, from evolutionary theory to genetic engineering, with their fictional prognosticators / counterparts, Bulwer-Lytton, Wells, Stapledon and Huxley. The short text scarcely can do more than sketch in a few pages either the biological or the literary issues, and historians of the science-fiction form would perhaps find it disappointing; nor does it attempt any theoretical understanding of the science/literature relationship or disjuncture. Instead, it is a version of the familiar exercise of 'enriching' the otherwise presumed-acultural science or engineering student. Nonetheless, it is an interesting attempt, and it will be well worth discovering how students fare with it.

The remaining two books in the series are less adequate. Neither Ernest Braun and David Collingridge (*Technology and Survival*) or Keith Pavitt and Michael Worboys (*Science, Technology and the Modern Industrial State*) seem to have given much thought to the specific problems of presenting texts in this type of format. The writing is didactic and students are not given much chance to think for themselves or develop a critical approach to the material offered. Braun and Collingridge admittedly have a tougher task if only because the questions of pollution, population and resource depletion have been so much under public discussion in recent years, but their bald treatment, for example, of the issues raised by the *Limits to Growth* approach, leaves much to be desired even in so brief a booklet. Pavitt and Worboys' sterile and telegraphic style is simply inadequate to their theme; and if space only allows, say, three and a half pages for science and technology in the US since 1945, and six for Western Europe, it is difficult to see the point of the exercise. The student could be instead directed immediately to one of the original texts on which they draw.

Despite the high hopes with which the SISCON exercise was launched, it is hard

not to feel a certain sense of disappointment. The booklets are quite nicely produced, except for the irritating use of unjustified right-hand margins and a quite unnecessary adoption of American spelling throughout (as most of the exemplary material is British or European one presumes that the American student market will use the books on their academic and pedagogic merit, not on the spelling of labour as labor). But the series lacks a sense of intellectual excitement, of critical bite, and the choice of

topic does not have an obvious coherence. To create teaching material of the SISCON type was a challenge not merely to the individual booklet authors, but to the central editorial board and its editor-in-chief, Dr Williams. Surveying the first products of their labours, it is evidence of this central editorial function which above all, seems lacking. □

Steven Rose is Professor of Biology at the Open University, Milton Keynes, UK.

Mathematical biologist's pot-pourri

Most really interesting problems in biology are dynamic, complex, and difficult to solve by intuition and experiment alone. Mathematical models are not a substitute either for doing experiments, or for sound intuition. However, used properly models provide the third leg without which efforts to understand complex biological systems, like milking stools, tend to fall down.

Modelling has a long tradition in biology, but until comparatively recently, few practitioners. Now it seems, almost everybody wants to do it; and with it come the books to help them. Unfortunately, if you merely teach a biologist some mathematics, or worse, a mathematician some biology, you are as likely to get Mumbo Jumbo as magic. Good modelling is an art, in which intuition and flair play a major part. You cannot teach either of these but you can nurture and guide them when they develop.

There are now several books from which biologists can learn mathematics—the nuts and bolts of the job. They are, with respect to the authors, probably the easiest to write because all they have to do is take established mathematical techniques, shuffle them intelligently, and illustrate them with appropriate biological carrots. Evaluation and criticism rest on the choice of techniques (it will be impossible to please everybody) and how well they are explained (which is difficult to judge once you know the answer). *A Biologist's Mathematics*. (Arnold: London, hardback £9.95; paperback £4.95), by D. R. Causton, has been written for first- and second-year undergraduates, and assumes only a very elementary knowledge of mathematics; how elementary can be judged by its opening chapters on numbers, indices and logarithms (chapter 2), multidimensional space (chapter 3) and functions and curves (chapter 4). The 'real mathematics' (that is, the point at which many of my own students begin to have difficulties) start in chapters 5 and 6, which deal with differentiation. Subsequent chapters run

through integration and the solution of differential equations, series, trigonometrical functions and matrix algebra. If these are the sort of techniques that you would like your students to know about, then this is a reasonable little book to recommend. Some idea of the level that they will achieve by using it can be gained from the fact that the chapter on matrices discusses determinants, but not eigenvalues, and those on calculus make no mention of partial differentiation. In other words, any student who grasps most of its contents will have a sound mathematical foundation to build on, but would still find great difficulty with chunks of the book by Pielou, reviewed later.

Nor would he or she be much of a modeller. To model successfully requires more than a mixture of biology and mathematics, a point of view that is developed most elegantly and forcefully by H. J. Gold in *Mathematical Modeling of Biological Systems: An Introductory Guidebook* (Wiley-Interscience: New York and London, £15.60; \$27.90). This is an excellent book. Its basic premise is that mathematical procedures are useful and necessary for the description and understanding of biological systems. Its primary object is not to teach the mathematics, but to help the student understand the relationship between the biological system under study and its mathematical description. As such it is very definitely a graduate and senior undergraduate text, and one that many more senior biologists could gain a lot from reading. I did.

It draws its examples from a wide spectrum of biology; the growth of bacterial populations; biochemistry and cell metabolism; biological rhythms and clocks; foraging bees; the spread of pollution; population regulation; and predator-prey interactions. My only regret is that its main example, used to illustrate several key steps in the book, is not biological but electrical, namely the design and functions of a public address system. Even the simple electronics involved will not be familiar to many biologists, and hence the student is faced with the dual problem of learning new concepts based on an unfamiliar example.

The book deals thoroughly with such elementary (but potentially confusing)

concepts as parameters, inputs, outputs, state variables and feedback. It describes the difference between stochastic and deterministic models, starting from the (correct) premise that most biology students have never had any formal training in probability theory. For similar reasons, it devotes a whole chapter to dimensions, and the booby-traps that await the unwary if we forget about them, or worse, don't know about them. And it is at pains to distinguish between continuous-time and discrete-time models, and when one can and cannot use differential equations to describe biological systems.

The emphasis is on constructing fairly simple models that can be solved analytically, the main theme of the book leading to two chapters (6 and 7) which deal with the assembly of compartmental models and the analysis of their dynamical behaviour, although (in keeping with the rest of the text) the mathematics of formal stability analysis are not presented in detail. The use of computers and simulation to analyse more complex models is discussed in chapter 9, which also touches on numerical taxonomy.

These brief comments do not really do the book justice. It has a series of valuable appendices, is packed with worked examples, hints, techniques and sound common-sense. What is more, it is rigorous and not afraid to develop mathematical arguments fully where need be. Two messages in particular are repeated throughout. Models are only as good as the assumptions that go into them; and if the output departs dramatically from one's biological intuition, you are as likely to have got the model wrong as to have won a Nobel prize. It is essential reading for all would-be modellers.

I am less certain about the third book in this quartet, edited by C. A. S. Hall and J. W. Day, Jr: *Ecosystem Modelling in Theory and Practice: An Introduction with Case Histories* (Wiley-Interscience: New York and London, £21.25; \$38). It is much longer than Gold's (I am tempted to suggest that there was not time to produce a smaller book), and is much less informative, page for page. To be fair, its objectives are slightly different; it deals only with ecology, and then only with large-scale computer models, rather than simple models that can be handled analytically. But it does overlap sufficiently to look poor by comparison. It too is aimed at senior undergraduates and immediately postgraduate students.

Like most multi-author texts, it suffers from a certain lack of cohesion, changes of terminology and symbolism that more vigorous editing should have eliminated. The chapters are also very variable in quality. It is divided into two main parts. Part 1 is entitled "Basic Principles" and its first four chapters overlap considerably with Gold's book, but do it less well. The exception is chapter 4, by Schoemaker, which is excellent. The remaining intro-

ductory chapters are novel, dealing with the sociology and politics of building models to solve environmental problems, basic economics and the use of models in cost-benefit analysis, together with an attempt by H. T. Odum to coin a whole new set of economic values in terms of energy.

The second main part of the book consists of case histories: 17 individual research papers that describe simulation models of particular ecosystems. They fall into three parts. Four chapters are studies of natural ecosystems, followed by six directed toward assessing the impact of large-scale environmental changes on various communities (or in one case the biosphere; not surprisingly, this model didn't work). The last seven chapters to some extent overlap with the middle six, and consider optimum management strategies for ecosystems using simulation models.

Perhaps I'm funny, but with one or two notable exceptions, I find reading about other people's detailed computer models boring; hardly any general principles ever seem to emerge. Rather, two things come across very strongly. First, large-scale models are much more useful for solving local, tactical management problems than they are for generating interesting (that is, general) ecological insights. Second, fruitful contacts between those who wish to generalise using simple, analytical models, and those who construct very detailed computer models of particular, complicated systems are depressingly few. Just three papers manage to bridge this gap in the present volume; by Botkin, on forest succession; by Wiegert, on thermal-spring food-chains; and the second contribution by Schoemaker, on pest control. It probably isn't coincidence that none of them try to model an entire, complex ecosystem, but instead concentrate on interactions between small sets of species. This seems to me to be the most hopeful (and indeed exciting) level to try and weld together these two very different approaches to ecological modelling.

In short, Hall and Day could provide useful background reading for a student wanting to find out what large-scale computer modelling is about. But he would be wise to select what he reads carefully (lest like me he falls asleep!), and to heed Gold's warning that "there is no easy cure for this form of 'numeromania'".

The final volume in this *pot pourri* for mathematical biologists, by E. C. Pielou, is the second edition of a familiar and successful text: *Mathematical Ecology* (Wiley-Interscience: New York and London, £12.30; \$23.50). I can therefore be brief. Significantly, the words *Introduction* to are missing from the title of the second edition. It is as well. This book is not for the mathematically faint-hearted. The treatment is rigorous and advanced, and in order to cope students will need rather

more than just Causton's book; in fact they will need to be thoroughly at home with calculus and matrix algebra, and to have had a good grounding in statistics.

The book picks selected topics over the whole range of mathematical ecology, following the outline of the first edition closely, though with numerous minor and two major changes. Chapter 6 now contains a brief discussion on the place of mathematical model building in ecology; these four pages should be compulsory reading for all students who have a desire to play with models. I am less sure about the other major change which is an entire chapter (17) devoted to the statistical analysis of zonation. As a 'natural experiment', zonation is important, and the need to actually look at what happens in the field cannot be re-emphasised enough. But it is a pity that the chance was not taken to use simple analytical models (or simulations) to interpret, and create hypotheses about particular patterns of zonation. Instead, reference to Pielou's own stimulating work in this area is buried 150 pages previously, with the result that most of the potential interest of

this new chapter will probably be lost on most students.

This is a detailed quibble. The second edition of Pielou's book taken as a whole re-affirms its unique status as a rigorous and comprehensive summary of 'two broad and very different areas of mathematical ecology: the first (in chapters 1-6) dealing with the construction and exploration of analytical models of traditional ecological problems (population dynamics, competition, predator-prey interactions, and so on); and the second (chapters 7-22) with the statistical description and analysis of ecological events as they occur in the field. From this second very large area, which we may roughly call 'statistical ecology' arise major questions that need to be answered by the first. No other book combines both aspects of mathematical ecology.

For advanced ecology students, at least, a combination of Gold and Pielou would provide a formidable training, as well as a great deal of valuable reading for their teachers.

John Lawton

John Lawton is Lecturer in Ecology at the University of York, UK.

Population dynamics

Analysis of Vertebrate Populations. By G. Caughley. Pp. 234. (Wiley: London, New York and Sydney, 1977.) £8.75; \$16.50.

THIS book is a valuable addition to the literature on population dynamics, both for students who are hoping to devote their careers to wildlife management and for those who are already involved in it and are struggling with statistical textbooks too broad and mathematical for their needs. Other students who are interested in population processes in a more fundamental way will find its lucid lay-out and exposition a painless introduction to deeper matters.

As the dust-jacket and the author's career indicate, the emphasis of the book and of its examples is on populations of large mammals, especially ungulates. However, his wide reading among and critical assessment of researches on other groups of vertebrates widen the application of the principles discussed. The author achieves, from the starting point of mammals and birds, the same valuable generality as did T. R. E. Southwood, starting from insects, in his *Ecological Methods* (1966).

From the point of view of the student whose ecological interests stem from a Natural History background (and these are in the majority), Dr Caughley's great virtue is that his feet remain firmly planted in the field. He exhibits the late David Lack's maxim of "know your

animal" to the n th. degree. Thus, time and time again, he is able to point to instances where strict adherence to standard statistical tests makes biological nonsense and usually to suggest a modification or short cut which gives a reasonable approximation to the truth. Above all, he has a sympathetic understanding of the shortcomings of data gathered (often with great dedication) in the field, so that any student, who has absorbed this book, will return to the field with new eyes as to what he can and cannot achieve in his special sphere of interest.

The chapters follow a well-planned sequence: what is a population and what measurements describe it; the importance of age in these analyses; how do we estimate abundance, rate of increase, dispersal, fecundity and mortality; how are these parameters integrated to produce observed results; and, finally, how do such analyses direct us in designing measures of conservation, cropping and control? The reader is not plagued with complex mathematics; a knowledge of basic algebra and statistics is the only equipment needed.

Errors are remarkably few; the only notable one I could find was on p60 where *Microtus oeconomus*, the root vole, is referred to as the bank vole which is in a different genus (*Clethrionomys*). The standard of production, generally, is high and the straightforwardness of the writing admirable.

H. N. Southern

H. N. Southern recently retired as Senior Research Officer of the Animal Ecology Research Group at the University of Oxford, UK.

Microbial biology

MICROBIAL ecology is a difficult subject to teach, being concerned with complex biological interactions and relying on diverse expertise for its investigation. Dr R. E. Campbell in his book *Microbial Ecology* (Blackwell Scientific: Oxford, paperback £4.25) states that he has chosen to view the subject in terms of communities of interacting organisms, rather than form a physiological standpoint. Having made this point, he then proceeds to an excellent short survey of microbial ecology, which by its very nature must include detailed consideration of physiological processes. The testing of reactions in the carbon cycle (p21), the discussion of cellulose metabolism (p25), and the accounts of nitrogen fixation (pp44–45) and of the activities of microorganisms in sulphur conversions (p55), are only a few of the many physiological and biochemical topics whose significance in an ecological context is clearly defined by the author.

The book is divided into nine chapters. The first two are introductory; these are followed by three on the carbon, nitrogen, sulphur and other chemical cycles. The structure and dynamics of microbial populations in soil, water and air are covered in three chapters, and there is a final short conclusion. Within this compass, Dr Campbell has been able to include almost all that most microbiologists would expect in an introductory text.

The first chapter, a short introduction, includes a section on methods of study (plating, ATP and so on) and on modelling. Both can only titillate the more motivated student. "Ecosystems are never static", the first sentence of chapter 2, leads the reader directly into the basic concepts of energy flow, food chains and trophic levels, which are covered without enough specific examples to my mind, but are presented in a chatty style. If you like this approach, the chapter will please. I felt more at ease in chapters 3, 4 and 5, in which Dr Campbell describes the various chemical cycles (carbon, nitrogen, sulphur, phosphorus, iron, manganese and silicon). Students will undoubtedly find the clear figures and diagrams useful. Figure 4.1, the global nitrogen cycle, and Table 5.1, sulphur conversions, are particularly good, although the print is a little small in some places (for example, Figure 4.5). The degradation of manufactured materials, and biocides in the carbon cycle, are also discussed in two short sections.

Chapters 6, 7 and 8 concentrate on the diversity of types of naturally occurring microorganisms in soil, water and air. In these chapters enough ecological background is presented for the novice reader to understand why particular microbiological investigations are important.

As a single example, the significance of soil water supplying nutrients and possibly causing anaerobiosis is discussed on p64. There are also interesting discussions on the rhizosphere around plant roots, biological control of soil-borne pathogens, eutrophication and pollution, air inside and outside buildings, and the microbiology of animal surfaces.

Personally, I like the book, and know of no other having such a broad coverage and simple presentation. For the serious student, there are reading lists at the end of each chapter, and comprehensive species and subject indexes; he has, therefore, no need to complain that the subject treatment is too superficial. The book is excellent value.

Professor M. Alexander has produced a second edition of his *Introduction to Soil Microbiology* (Wiley: New York, London and Sydney, £13.50; \$23) which he has brought up to date and also modified by considering how soil microorganisms cope with the effect of man (pesticides, pollutants), and what toxicants they produce in soils. The dust-cover considers the book to be an introduction and "ideal for beginners"; however, its cost and length (467 pages), suggest that it should be considered more properly as an advanced undergraduate or postgraduate text. Detailed reading proves this to be so, and one could quote many examples. There are, for instance, ten pages devoted to hemicelluloses besides fourteen on celluloses, and an advanced account of the biochemistry of hemicellulose decomposition (pp171–172), all of which are hardly ideal for beginners, although first-rate expositions. Having said this, the book is an excellent source for the specialist or advanced student: I

was not well informed on the acetylene reduction technique for nitrogen fixation and found it summarised on p288, nor was I aware of modern views concerning lignin decomposition which Professor Alexander discusses fully.

The book is divided into five parts. The first is a basic description of the taxonomic groups of microorganisms found in soil. The second and third are detailed treatments of the carbon and nitrogen cycle, and cover 190 pages. There are many important topics included here, such as the microbiology of polysaccharides, nitrification and organic matter decomposition. It is possible to argue that the chemical pathways of benzene ring cleavage (p219) and purine metabolism (p236) are available in standard chemical texts; however, many students will find their inclusion helpful. There follows a section on mineral transformations in which the metabolism and fate of phosphorus, sulphur, iron, and heavy metal pollutants are considered. I was particularly interested in the discussion of mercury, selenium, and arsenic in soils. The final part of the book is called "Ecological Interrelationships" and includes chapters on interactions among species, the rhizosphere, and pesticides.

In general, I found Professor Alexander's book a well written advanced text on soil microbiology with excellent literature references, and can recommend it as a straightforward account interlaced with many references to areas of doubt that should suggest research topics to the motivated student or postgraduate.

P. S. Meadows

P. S. Meadows is Senior Lecturer in Zoology at the University of Glasgow, Scotland.

Plant pathology

Plant Pathology and Plant Pathogens. By C. H. Dickinson and J. A. Lucas. Pp. 161. (Blackwell Scientific: Oxford, London, Edinburgh and Melbourne, 1977.) £4.25.

THIS introductory plant pathology textbook adopts the approach of a general treatment of principles without detailed consideration of individual diseases. An appendix is included giving cross references to other books for this detail. An adequate microbiological background must be assumed, although the authors have sometimes defined simple terms such as mycelium and given little or no explanation of more difficult ones such as teleutospore.

The text moves from general introduction to infection and colonisation, host-pathogen interactions from population to molecular levels, and finally control. Physiological and molecular interactions are treated in some detail, even in relation to still unresolved problems, such as the concept of hypersensitivity. Conversely,

well established areas such as epidemiology and chemical control are treated relatively superficially. The authors tend to undervalue the contribution of the agrochemical companies to successful disease control and to the avoidance of toxicological and environmental hazards. The "Integrated Control" section, on the other hand, hardly warrants the prominence given it in the publisher's note. The text is well illustrated with diagrams and electron micrographs but has few illustrations of pathogens or diseases of the kind that would help the student to develop a feeling for what plant diseases are like.

These considerations apart, however, the book is clearly, concisely and interestingly presented and helps fill the gap between the short Institute of Biology *Studies in Biology* type of format (Arnold: London) and the heavy textbook. Undergraduates should find it useful, especially if they combine it with a study of individual diseases from other texts or from practical work.

I. M. Smith

I. M. Smith is Lecturer in Plant Pathology at Imperial College, London, UK.

Introductory parasitology

IN *Biochemistry of Parasitic Protozoa* (Macmillan: London, hardback £10; paperback £4.95), W. E. Gutteridge and G. H. Coombs have succeeded very well in their intention—to produce a readable introductory text for people coming to the subject without special preliminary grounding in either protozoology or biochemistry. It is aimed at undergraduates and first-year graduates, but it will be useful to protozoologists in general, for quick consultation in areas of protozoal biochemistry with which they are not immediately familiar. The text is concise, lucid and systematic, placing emphasis on the significance of the studies described, and particularly on parasite/host metabolic differences which may possibly be exploited for chemotherapeutic and immunological purposes.

There are ten chapters dealing with: energy generation in the vertebrate and in the invertebrate host; metabolism of nucleic acids, proteins and lipids; drug action; and possible future developments. There are helpful appendices dealing mainly with the preparation of organisms for biochemical study and with the use of the literature. The index is restricted on purpose, as the contents of the book are systematically arranged and cross-referenced.

The only minor suggestion to make for a later edition is to include a short section on the significance of the conventions of biochemical diagrams: what are meant by all the tangential arcs, NADHs and ATPs in boxes and not, and so on?

The basic textbook by G. D. Schmidt and L. S. Roberts, *Foundations of Parasitology* (Henry Kimpton: London, hardback £15.85; paperback £11.80) is intended for introductory courses in parasitology and seems to be aimed principally at final-year Zoology undergraduates.

The first two chapters outline the scope and basic principles of parasitology. This is followed by eight chapters on the Protozoa; an introductory chapter on form, function and classification; and seven others dealing in turn with the Kinetoplastida, other flagellates, the Sarcodina, the gregarines and coccidians, malaria and piroplasms, the Myxospora and Microspora, and the Ciliophora. The remaining 23 chapters deal with metazoan parasites (ten on the platyhelminthes, nine on the nematodes, and a chapter each on the mezozoans, acanthocephalans, pentastomids and parasitic crustacea).

The text is interspersed by a total of 889 line diagrams, photographs and electron and photomicrographs. The majority of these are informative and well-chosen. However, because most of the diagrams have been taken unaltered from other

publications, there is a rather disconcerting range of styles; and the photographs of cleared, flattened, microscope-slide preparations of vectors are not very useful. The electronmicrographs in particular suffer from the quality of paper and reproduction process, but perhaps these are necessary sacrifices in an effort to keep the prices of textbooks within reasonable limits.

The book is fairly conventional in scope and treatment, although more comprehensive in its range than most equivalent textbooks (for example, the inclusion of the parasitic crustacea). There is a full treatment of all important parasites of human and veterinary importance, considered under the standard headings of morphology, pathology, epidemiology, and so on.

A large amount of information is presented, and although it will undoubtedly be a useful reference work for basic information, it is less useful as a source of guiding concepts on the subject. For instance, in the section on the Protozoa, although reference is made to other parasitic groups such as the viruses, bacteria and fungi as "disciplines in their own right", it is not pointed out that the Protozoa have strong conceptual resemblances to these organisms, multiplying in the body of the host, and dissimilarities from the helminths, which in general do not do so. A more important deficiency concerns the meagre reference to immunological concepts or to the importance of immunopathological mech-

anisms in the descriptions of the various infections. Although there is a useful introductory section on basic immunology, the text does not sufficiently reflect the new and exciting findings responsible for the recent widespread interest in parasitism due to protozoans and helminths shown by basic immunologists.

Although there are some errors, inevitable in a book providing such a wealth of detail on such a wide subject (for example, the amastigote form is not "definitive" for *Leishmania*; nagana is not a "disease" of African antelopes; the tail ends of microfilariae shown in Fig. 30-3 for differentiation purposes are almost all incorrect), in general the facts are accurate and the information up to date.

This work has much to recommend it as a successor to the excellent but rather dated Chandler and Read (tenth edition, Wiley: New York, 1961) as a basic textbook of classical information on the various groups of animal parasites. Whether it is preferred to the recent new editions of other textbooks of equivalent standard (for example, those by Noble and Noble, Olsen, and Smyth) will depend principally on the emphasis of the course.

W. H. R. Lumsden

W. H. R. Lumsden is Professor of Medical Protozoology at the School of Hygiene and Tropical Medicine, London, UK. This review was completed in Professor Lumsden's absence by R. Muller, Senior Lecturer in Medical Helminthology.

Nematode biology

Biology of Nematodes. By N. A. Croll and B. E. Matthews. Pp. 201. (Blackie: Glasgow, 1977.) £6.25.

SEVERAL books on nematodes have appeared in recent years and this reflects the growing popularity amongst scientists of this widespread and exceedingly numerous group of animals. Some nematodes have long been recognised as parasites which cause serious diseases of man and his livestock. More recently they have been increasingly recognised as serious pests of crops. Enormous numbers of nematodes are, however, free-living in soil, and in marine and freshwater mud, but because these groups seem to be economically unimportant, are mostly microscopic in size and are taxonomically very difficult, they have tended to be ignored.

The *Biology of Nematodes* follows the trend of two other recent books on nematodes in attempting to treat nematodes as a group and not just as pests or parasites. The first chapter contains information on the taxonomic position of nematodes and a brief description of their structure. This is followed by a chapter on the genetics and ageing of nematodes,

their biochemistry, physiology, and the use of certain 'model' species in the study of parasitic diseases. The other chapters deal with neuromuscular physiology and sense organs, behaviour, feeding and nutrition, development (including hatching and moulting), types of life cycle, the biology of parasitic nematodes, survival, and the treatment and prevention of nematode diseases. Some of these chapters have adopted a new approach to the information and have covered the ground well, using several up-to-date apt examples.

Other aspects of the biology of nematodes are covered less well. For example, there is little or no mention of metabolism, osmoregulation or excretion. There are many photographs and electron micrographs which add to the appearance of the book but a lack of diagrams to complement descriptions of certain aspects, especially on the structure of nematodes, could make it more difficult for the reader who is new to the subject to fully grasp the information.

The book is easy to read, attractively produced and, at £6.25, is quite cheap.

D. L. Lee

D. L. Lee is Professor of Agricultural Zoology in the Department of Pure and Applied Zoology at the University of Leeds, UK.

Cellular genetics

SEX-LINKED recessive inheritance in haemophilia was known to the Jews by the time of Christ (they had to avoid circumcising males in certain families); Maupertuis, an astronomer, published pedigrees showing the inheritance of polydactyly in 1752; and Joseph Adams had described the differences between dominant and recessive inheritance, and the dangers of marrying close relatives by 1812. Despite all this, the significance of Mendel's work was not recognised until 1900, 35 years after he published it. The only lesson that can safely be drawn from this is that acceptance of scientific ideas often has to await the knowledge of underlying mechanisms: geophysicists had to discover the properties of tectonic plates before continental drift became 'respectable'; the control of protein synthesis by nucleic acids has in principle made genetics much more understandable to non-specialists.

Concepts of Molecular Genetics: Information Flow in Genetics and Evolution by D. O. and V. W. Woodward (McGraw-Hill: New York and London, £11.20) is an excellent interpretation of cellular events since the structure and role of DNA was unravelled. It is the sort of book beloved by students but shunned by teachers: it is written in 'chatty American' and uses a large number of clear diagrams to supplement the text. The main part of the book (373 pages) describes the background to our knowledge of DNA, and the importance of that chemical in directing the phenotypic consequence of chemical processes. It is highly readable, and even though the authors write about organisms (for example, *Neurospora*) as if they were chemical machines, they explicitly warn against the dangers of reductionism and, better still, treat protein evolution as the result of adaptation through natural selection rather than the chance mishaps of a clumsy clock. The book is slightly marred by a final chapter (35 pages) on "Heredity and Society" which would be a naive treatment of genetical influences in human society in any context, but is out-of-place at the end of an essentially biochemical text.

The Genetic Code by B. F. C. Clark is no.83 in the Institute of Biology's *Studies in Biology* series (Arnold: London, hardback £3.20; paperback £1.60). Clark is Professor of Biostructural Chemistry at Aarhus and a former colleague of M. W. Nirenberg, one of the significant figures in the elucidation of the genetic code; he will probably be confused in many minds with the geneticist B. C. Clarke of Nottingham, who has also written on the genetic code. This book covers much the same ground as that of the Woodwards in a briefer and more formal fashion. The author claims an aim "to convey to the

reader the experimental details which gave rise to our knowledge in this field", and it may be this that makes his text distinctly less readable than the larger book reviewed above. Nevertheless, it will be a useful summary for undergraduates of the main results in molecular genetics.

Chromosome Genetics by H. Rees and R. N. Jones (Hardback £10.50; paperback £5.25) and *The Genetics of Recombination* by D. G. Catcheside (Hardback £11.50; paperback £5.75) are two of the first three books in a new series titled *Genetics—Principles and Perspectives* being published by Arnold (London) under the general editorship of K. R. Lewis and Bernard John. They are very different in character. *Chromosome Genetics* is a stolid account of the structure and behaviour of chromosomes at both the biochemical and structural level. It is informative and honest (for example, "the mechanics of interference are by no means clear"; "the role of heterochromatin in gene regulation . . . remains conjectural"). The authors are botanists, and this produces certain biases in their treatment: they are good on B chromosomes but thin in their treatment of centric fusions. Nevertheless, their book will be a useful addition to many personal libraries, and a necessity for both school and university libraries.

Professor Catcheside's work is much more specialised. He writes mainly about

the exchange of genetical material in fungi, although he descends to phage and ascends as far as *Drosophila*. The result is a lengthy but not (in terms of his title) exhaustive review. For example, nothing is said about recombination in mammals, even though the seventeenth chromosome of the mouse at least is currently presenting some intriguing problems in the context of recombination. This is a book which will be of value to microbial geneticists, but of only marginal interest to others in the genetical gaggle.

Finally, the third edition of M. J. D. White's *Animal Cytology and Evolution* (Cambridge University: Cambridge, London and New York) published in 1973, has now been issued in paperback at £8.95. This is wholly to be welcomed. The book has been one of the more important biological syntheses produced in the modern era. First published in 1945, a re-written second edition of 454 pages was produced in 1955 at £2.25, and then another edition (again re-written and containing 961 pages) in 1973, this time priced at £25. If this paperback reprint means more biologists (all levels) read the book, nothing but good can result. Indeed, how often do publishers' prices in times of library economy mean that science is hindered? **R. J. Berry**

R. J. Berry is Professor of Genetics in the University of London at the Royal Free Hospital School of Medicine, London, UK.

Basic immunology

A NUMBER of basic texts in immunology have been published in the past year. *An Introduction to Immunology*, edited by E. M. Lance, P. B. Medawar and E. Simpson (Wildwood House: London, £4.95), is intended for biologists and clinicians who have no previous knowledge of immunology and who wish only to gain a passing acquaintance with the subject. It contains an introduction and five chapters, written in essay style, on immunoglobulins, lymphocytes and the lymphatic system, transplantation immunity and tolerance, tumour immunity, and immunological disease. The authors have aimed to produce an easily readable text and intend that the reader should be able to imbibe the contents one chapter at a time. Although the authors have mostly achieved their aim, there are a few minor criticisms which should be made. The print is very large but closely set on small pages; this makes for lack of legibility. The topic of cortisol sensitivity of thymocytes appears with no explanation and might confuse a non-immunologist. "Autochthonous", although defined on p104, is first used on p100, and ambiguous phrases such as ". . . certain dosage levels . . ." (p98) are too common. In conclusion, although this book will no doubt achieve deserved popularity, I hope

it will be presented with a more legible format in subsequent editions.

The Biology of Ageing by Marion Lamb (Blackie: Glasgow, £5.75) is an excellent account of gerontology aimed at undergraduates, but it could be recommended to any biologist who might require a general grounding in the subject. The text covers manifestations of ageing, statistics of ageing in populations, variation in longevity between different species, attempts to modify the rate of ageing, studies of ageing *in vivo* and *in vitro*, and hypotheses proposed to explain ageing. In reading this book one gains a strong impression that gerontology is paradoxically a vigorous field in which at the moment there are more questions than answers. Although this gives rise to much controversy, the author has presented a balanced and comprehensive view of the topic. There is also a useful bibliography for each chapter, thus enabling the reader to follow up specific topics in more detail. The publisher is to be congratulated no less than the author, as the text is legible, the figures and tables are clear and the cost is reasonable. An excellent text that doubtless will be used extensively by biology students.

Cellular immunology has proved a most difficult area of immunology to present in a fair and comprehensible manner. *The Cellular Basis of the Immune Response* by E. S. Golub (Freeman:

London, £6.50) has succeeded so manifestly in making cellular immunology comprehensible as to make one wonder what ever was the problem. The twenty short chapters are divided into four sections. The first, on lymphocyte populations, deals with the origin and distribution of lymphoid tissue, cell interactions in antibody formation, cell interactions in cell-mediated responses, properties of B and T cells, and the major histocompatibility complex. The second section is the best part of the book and deals with mechanisms of cellular co-operation in the immune response. The six chapters of this section cover effector and helper cells in antibody formation and in cell-mediated responses, mechanisms of effector cell helper cell co-operation, receptors and signals, the role of the macrophage, and development of B-cell function. In the third section, one senses a potential dilemma which the

author has dealt with fairly. The subject of this section is immunoglobulins, and although it is not an exhaustive coverage, sufficient information is provided for an immunobiology course. The fourth section covers immunoregulation in four chapters on proliferation and maturation of cells in the immune response, regulator T cells, the genetics of the immune response, and immunological tolerance. The presentation of the material is good and the clear simple figures contribute greatly to the elucidation of complex experimental designs. This is a first rate book of which the greatest virtue is its step by step approach which will aid immunobiology students greatly.

T. D. Heath

T. D. Heath is a research fellow in the Division of Biology of the Institute of Cancer Research working at the Chester Beatty Research Institute, London, UK.

Fascination of genetics

SEVERAL textbooks of genetics were published in 1977, each appealing to different tastes.

I. H. Herskowitz's *Principles of Genetics* (Collier Macmillan: London, second edition, £12.75), one of the standard textbooks of genetics, is about twice the size of the author's *Genetics* published in 1962. This is clear evidence of the considerable increase in our knowledge of the subject over the past decade. But there has also been some shift of emphasis, for now the genetics of man is given more prominence than previously. This is essentially a complete course textbook of genetics covering the entire field at the undergraduate level. Each chapter concludes with a section summarising the main points, and general and specific references; and, as in the author's other books, photographs of eminent geneticists are included for interest. There are also questions and problems, with detailed answers at the end of the book, an exceptional number of diagrams and illustrations, a glossary and an excellent index. To my mind this is one of the best undergraduate textbooks of general genetics currently available.

D. L. Hartl's *Our Uncertain Heritage: Genetics and Human Diversity* (Blackwell Scientific: Oxford, £11.20), is based on a course the author has taught to undergraduates in the humanities and therefore assumes the reader has little background knowledge of human biology. It follows the traditional topics and style of similar texts with sections on Mendelism, chromosomal abnormalities, and the basics of biochemical genetics and population genetics. Possibly as a *souçon* for those interested in the more social aspects of human biology, the author has inclu-

ded chapters on cancer, race, human origins and genetic engineering.

Each chapter in Hartl's book concludes with references for further reading but these are often sadly out of date (the most up-to-date reference on prenatal diagnosis is 1971). The illustrations are generally good but the time-honoured Royal pedigree of haemophilia, which the author discusses at some length, is incorrect. There is not a great deal which makes this textbook any different from many others from which it will have very strong competition. I still feel that the best textbook of human genetics currently available for non-science socially-orientated students is Lerner and Libby's *Heredity, Evolution and Society* (Freeman: San Francisco, second edition, 1976, £8.80).

Genetics Study Guide by M. E. Conklin and D. L. Hartl (Lippincott: Philadelphia, Pennsylvania, paperback \$5.95), is quite a different type of book and, in many ways, unique. The authors believe that "... merely reading about genetics is quite a different matter from truly understanding how the laws of heredity operate"—genetics has first of all to be clearly understood. It is to this end that the authors have produced this book, which consists of 23 chapters ranging over the entire field of human genetics. Each chapter has a glossary of terms followed by questions and problem-solving exercises. There are also four crossword puzzles designed to help the student learn the vocabulary of genetics. The *Guide* is meant to accompany an introductory textbook of genetics (such as Hartl's own book). The intention throughout is to make the student an active participant in the learning process. Unfortunately, as no answers are provided, this is not a self-instructional text and would require the answers to be submitted to a teacher—presumably the reason for the pages being perforated so that they can be

easily torn out. If the intention is for the student to teach himself, then a self-instructional or programmed text (such as A. Vegotsky and C. A. White's *A Programmed Approach to Human Genetics*, Wiley: New York, 1974), might be considered preferable to a *Study Guide* which would seem superfluous to a good teacher sensitive to the problems and needs of his students.

It is becoming increasingly clear that many important hereditary characters both in man and other organisms are not inherited in a simple Mendelian manner but show continuous variation with no sharp distinction between different classes. Biometrical genetics is the study of such continuous variation and K. Mather and J. L. Jinks' *Introduction to Biometrical Genetics* (Chapman and Hall: London, hardback £8.50; paperback £4.50), is a worthy successor to their more comprehensive *Biometrical Genetics* which was intended for the specialist. Their new book is directed more toward senior undergraduates. In general, no more than simple algebra is needed, though on a few occasions, such as in discussing additive and dominance effects, the authors resort to matrix inversion. Rather idiosyncratically, *T* is used for heritability and not the usual h^2 which is used for something else. Examples are chosen almost exclusively from plants and animals and despite the authors' admission that "... man offers many advantages for the study of populations", biometrical methods in human genetics are dealt with in a mere dozen pages. It would certainly be useful to have an introductory text on the biometrical genetics of man particularly because of the importance of continuous variation in many medically important traits.

Finally, there is E. Novitski's *Human Genetics* (Collier Macmillan: London, £10.50), intended to interest the more junior undergraduate in the subject but at the same time provide him with some understanding of the many problems facing humanity from developments in this field. Thus, detailed discussions of population and biochemical genetics are sacrificed in order to place greater emphasis on the effects of radiation, transplantation and such socially important issues as the genetics of intelligence and race, genetic counselling and prenatal diagnosis. Each chapter concludes with a list of references, though very few are later than 1973, and a list of questions. It is clearly and attractively laid out and well illustrated with line drawings and many interesting photographs.

Each book will appeal to readers with different backgrounds and interests, which presumably is in part a reflection of the fascination of genetics.

Alan E. H. Emery

Alan E. H. Emery is Professor of Human Genetics at the University of Edinburgh, Scotland.

Biophysics texts

An Introduction to Biophysics by C. Sybesma (Academic: New York, San Francisco and London, \$19.50; £13.85) is written to fill a need for a comprehensive but elementary textbook on biophysics. In this it succeeds admirably. Sybesma writes from the standpoint of a physicist who is curious to know about biology, and about the problems that physics can help to solve. His curiosity extends across many of the boundaries of natural science. After an introduction to cell structure and biochemistry, the book moves on to various physico-chemical methods for looking at macromolecules, the origins of chemical bonding, photochemistry, biological energy conversion, the physiology of the nervous system and the anatomy of the ear, and ends with an almost philosophical chapter on theoretical biology.

The book should be readily comprehensible to physicists and chemists. For biologists there are appendices on quantum theory and thermodynamics. The approach is not over-mathematical, though some knowledge of calculus is assumed. In such a short book, one expects something must be skimmed; but as far as I can judge, the concepts of the various disciplines are patiently and thoroughly explained.

Cell Biology, by E. J. Ambrose and D. M. Easty (Thomas Nelson: London, Toronto and Sydney, paperback £5.95) also touches on a wide range of topics. In this second edition, a number of new sections have been added, notably on plant development. However, some subjects are covered much better than others. The sections on cell surfaces and embryology are full of good things; but those on bioenergetics, nuclear DNA and cell motility do not reflect the present state of research. Some subjects (physical methods for studying macromolecules, for example) are covered at such breathtaking speed that the biology student would have difficulty in grasping their significance. The book is well illustrated (readers who experience a sense of *déjà vu*, note that the same figure is shown on pages 164 and 230). It should make a useful contribution to a course in cell biology, but not as the sole textbook.

Despite its title *Cells and Energy* by R. A. Goldsby (Collier MacMillan: London, second edition, paperback £3.75) is not principally concerned with cell biology or bioenergetics, but with straightforward biochemistry. There are now some excellent textbooks which give a comprehensive coverage of biochemistry, but the amount of information may be overwhelming to students who only need a taste of the subject. This book adopts a more 'popular' approach. The

first chapter is on the origin of life, chemical evolution and the prospects for intelligent life elsewhere in the Universe; it does not deal, however, with the evidence for the earliest living organisms on Earth (which is described by Ambrose and Easty). Then on to the biochemistry proper, where a good selection of topics is introduced, with occasional diversions on special topics such as the way in which electron micrographs are made. Students should find it a readable introduction to the subject.

In *Energy and the Living Cell: an Introduction to Bioenergetics* (Lippincott: Philadelphia, Pennsylvania, \$7.95), W. M. Becker takes the subject of his title more seriously. A sound basis is provided by early chapters describing thermodynamics and enzyme kinetics (the latter explained in terms of monkeys and peanuts—a description that the author clearly relishes). The following chapters describe how cells obtain energy by photosynthesis, respiration and fermentation, and how cells use energy in biosynthesis, concentration gradients across membranes, and muscle contraction. The concepts involved are explained very clearly and each chapter ends with a useful set of practice problems. Unfortunately some of the recent developments in bioenergetics are not mentioned; for example, the picture of the mitochondrial respiratory chain has an old-fashioned look about it, with FAD treated as a free nucleotide, and iron-sulphur proteins nowhere to be seen.

Transport processes through biological membranes

Cell Membranes and Ion Transport: Integrated Themes in Biology. By J. L. Hall and D. A. Baker. Pp. 127. (Longman: Harlow, UK, 1977.) £3.50.

THERE is obviously a potential role for a simple student text on the significance and mechanisms of transport processes through biological membranes, and *Cell Membranes and Ion Transport* now joins M. Davies's *Functions of Biological Membranes* (Chapman and Hall, *Outline Studies in Biology*, 1973, £1.50) in this field. Hall and Baker say that they have aimed their text at first- and second-year undergraduates in biological sciences, though their publisher's cover comments extend this to "postgraduates who require a basic introduction to the subject".

The opening chapter on the nature of membranes is largely accurate but relatively pedestrian. Its electron micrographs are uninformative, it discusses a number of now defunct theories of membrane structure and it perpetuates the common myth that the major polar lipids of membranes form micelles when dis-

G. S. Campbell in *An Introduction to Environmental Biophysics* (Springer: Berlin and New York, paperback DM 20.20; \$8.90) deals with the micro-environment of living organisms. A bug sitting in the sun, a tree in a forest canopy, or a reader of *Nature*, all experience a microenvironment which is probably very different from the average climate described by the weather report. This has a profound effect on properties of the organism such as temperature and energy consumption. These properties may be difficult to measure; instead, the book describes methods of deriving them by calculation. The effects of variables such as air temperature, wind speed, evaporation and radiation are investigated by using models of energy and mass transfer similar to those used by engineers. Each chapter ends with numerical problems, which make fascinating reading: "What is the Reynolds number of an elephant?"; "How much food does a 500-kg caribou need to survive an arctic winter with average temperature -20°C , wind speed 3m/s ?". No answers to these questions are provided. In a way, this epitomises the difficulty inherent in the approach. Some day, a hardy field ecologist will have to go out on the frozen tundra and find out, the hard way, how much a caribou actually eats, just to make sure.

Richard Cammack

Richard Cammack is Lecturer in Biology at King's College, University of London, UK.

persed in water. Since the remainder of the book tends to concentrate on ion transport phenomena, rather than mechanisms, it is not entirely clear why much of this chapter is included.

The analysis of the kinetics and driving forces for transport which forms chapter 2 is a valuable element not found elsewhere in introductory texts: it includes both a classical kinetic treatment and a description of the application of irreversible thermodynamics to the study of interdependent transport processes. However, deficiencies include a "hyperbola" which rapidly reaches V_{max} (which should be an asymptote) and a mechanistically confusing description which presents energy deprivation of active transport as an example of non-competitive inhibition.

The third chapter considers mainly ionophores, ion selectivity by tissues and conceptual models of transport mechanisms. As in the membrane structure chapter, a variety of historically important models are offered, but this time there is little guidance as to which are closest to biological reality. Indeed, the authors give most emphasis to ideas of rotating and diffusing carriers, even though recent molecular evidence suggests that transport proteins are usually amphiphilic and span membranes (that is, they probably provide selective trans-

membrane channels).

Next comes a useful treatment of energy-linked transport processes in microorganisms, mitochondria and chloroplasts, punctuated by a discussion of ATP-driven transport (mainly of Na^+ and K^+). The latter part of this chapter is marred by terminological confusion: in particular, "counter-transport" is used to mean "antiport", a usage different from that earlier in the book.

Finally, there are discussions of algal ion transport and of Na^+ /organic solute cotransport (symport): these are designed to illustrate and integrate the concepts offered earlier. They do effectively round off the book in a physiological sense, but neither system yet gives much information on the membrane events involved in transport.

As the authors point out, their book will only work if students can integrate its parts into a coherent intellectual whole. My fear is that the authors' incomplete synthesis will only allow further successful integration by the brightest of students. This may be partly

because the book deals exclusively with ion transport, rather than with all types of solute transport through membranes. Even within ion transport, there are no detailed treatments of some of the systems that are most fully characterised in molecular terms (for example, sarcoplasmic reticulum Ca^{2+} -transport ATPase and the erythrocyte anion transporter). A possible problem throughout the book is the use of mol m^{-3} as the unit of concentration: although strictly correct, it will initially be alien to most readers and it therefore needs some introductory comment.

In summary, the need for a student text on transport is to some extent satisfied both by Hall and Baker (new and expensive) and by Davies (older but cheaper), but neither has really achieved the desired broad synthesis of current biochemical, biophysical and physiological ideas.

Bob Michell

Bob Michell is Lecturer in Biochemistry at the University of Birmingham, UK.

Developmental biology

Tissue Interactions and Development. By N. K. Wessells. Pp. 276. (W. A. Benjamin/Addison-Wesley: Menlo Park, California, 1977.) Hardback \$14.95; paperback \$9.95.

SINCE this book enters a competitive field, it needs something special to succeed, and it certainly has unique features. It's short: only about 100 of its 276 pages are generously spaced text, the rest being index, glossary, references (at the end of each chapter), summaries, diagrams and some beautiful scanning electron micrographs. After four introductory chapters, there follow fourteen on tissue interaction examples and mechanisms—limbs, skin, hormones, nerves, branching, cell contact, and so on.

It is intended to be read straight through. Different aspects of a system are treated at various points, and the lack of an overview can create difficulties. For example, limbs (including most mutants) are in chapter 6, with no hint of a role for cell movement. Then, in chapter 13, is Ede and Flint's *talpid*³ work, not put into the limb context nor listed in the index under limbs. The reference lists are unsystematic too, including only work that Wessells considers accessible and suitable as entries to a field. References are given with comments on usefulness, not titles; these seem to be well chosen, though a trifle daunting to junior students (a complete Cold Spring Harbor symposium!). This approach can be frustrating: for example, a student following up mention of nutrient effects on limb mesenchymal differentiation (p74) will find no reference

to Caplan.

The writing is often loose, creating misleading impressions: *Archaeopteryx* surely had fingers or digits on its wings, not toes (p67); mouse dermis and chick cornea together form feather buds, not feathers (p55); only *part* of amphibian mesoderm derives from gray crescent (p25); "molecules called intramembranous particles" (p183)?; gap junctions allow "rapid diffusion" and make "cells freely permeable" (p232)?; and so on.

I noticed few clear errors: Ede and Flint (not just Ede) suggest *talpid*³ cells are *more* adhesive than normals, not less (p193); the reference to Goss should, I think, be with chapter 12, not 16; and, a notable piece of self-effacement, a reference to Grainger and Wilt should be to Wessells. However, Wessells is often carried away by one side of an argument—on the interpretation of nuclear transplantation results, on the stability of the differentiated state, on the mechanisms of growth control and contact inhibition, on genetic restriction and expression as separate processes, and so on—though the references give access to other viewpoints.

The book has at least one serious omission: it clearly identifies the cell surface as a major site of cell interactions, but has nothing on lectins, glycosyltransferases, surface modulation or differential adhesion.

Despite the criticisms, I found the book lively. Its questioning approach should make it a stimulating supplement to standard texts.

J. R. Downie

J. R. Downie is Lecturer in the Department of Zoology at the University of Glasgow, Scotland.

Photosynthesis from the biochemical viewpoint

Biochemistry of Photosynthesis. (Second edition.) By R. P. F. Gregory. Pp. 221. (Wiley: London, New York and Toronto, 1977.) £8.50; \$16.50.

THIS is an up-dated and modified version of a book first published five years ago. In its original form it was unique: although there were other excellent text books on photosynthesis available there were none which tackled this subject from a biochemical viewpoint. The second edition enhances the earlier efforts of the author. He has made considerable changes, bringing the text in line with recent developments, and has deleted some of the superfluous material.

The book keeps the same basic format as the first edition. Part I concentrates on basic principles and concepts. This part of the book is composed of five chapters which essentially cover all aspects of photosynthesis presented, using a time-scale as a guide. That is, the first three chapters deal with the properties of light, its absorption by pigments, and its transfer to specialised photochemical reaction centres. The following chapters then outline the essential features of the energy conversion processes both at the electron transport level and at the subsequent carbon fixation stage.

The second part of the book serves to supplement and extend the fundamental and general concepts presented in the first five chapters. Here the author presents evidence for the Z scheme, covers chlorophyll and chloroplast organisation, and provides detailed and up-to-date discussions of electron transport and phosphorylation both for oxygen-evolving organisms and photosynthetic bacteria. The final chapter (chapter 10) deals with carbon metabolism and biochemistry of intact chloroplasts. For example, there is description of the role of the chloroplast envelope in controlling movements of substances into and out of the stroma as well as discussion about chloroplast autonomy.

This well presented and very readable book was written for undergraduate courses and I think it has greatly benefited from the changes which have been made. I thoroughly recommend the book and congratulate the author for producing a very worthwhile contribution to the teaching of this important and key biological subject. His inclusion of some numerical problems and questions, key references and an appendix of physical constants and formulae is valuable.

J. Barber

J. Barber is Reader in Plant Physiology at Imperial College, University of London, UK.

Advances in the life sciences

AMONG the many books published in the past year on the life sciences, three merit attention in these columns: one for the layman and undergraduate, one for use as a course textbook, and the other an advanced text on evolution.

The Life Science: Current Ideas of Biology (Harper and Row: New York, hardback \$8.95; Wildwood House: London, hardback £4.95; Granada/Paladin: London, paperback £1.25), to this reviewer, acted as both a stimulant and an irritant. The authors, P. B. and J. S. Medawar, take their readers on a conducted tour of life along, as it were, a series of stepping stones; each stone is explored in greater or lesser detail and features of importance, or just of interest, are described and popular misconceptions dispelled. Occasionally, as with the chapter on immunology, the area of exploration is larger and the insight gained is the more satisfying. The somewhat staccato nature of the progress from stone to stone highlights those areas or concepts which the authors regard as important, but has the drawback that it leaves the reader in ignorance of those stones which the authors have chosen not to tread upon. For example, set against a chapter on the genetical theory of evolution by natural selection is one on Lamarckism; this is the stuff of many schoolday essays, but totally ignored is the current, and fundamental, argument between neutralist and selectionist theories of evolution. But then, a book cannot be all-embracing, and it would always be possible to criticise what has been included or left out; the book has chapters on eugenics, demography, behaviour, microbiology, circulatory and coordination systems, cancer and senescence, to name but a few.

The careful, indeed precise formulation of the arguments makes for a very clear exposition and a very readable book; it also means that ambiguous statements or teleological (as opposed to telonomic) arguments are the more noticeable. Does the statement that "hermaphrodite animals such as snails and earthworms invariably cross-fertilize" imply that self-fertilisation does not occur in slugs, or just that they are not like snails? And a "final cause" is surely invoked in the passage "... the purpose of the pairing of sense organs is obvious enough. The pairing of eyes is the property that makes stereoscopic vision possible ...". A question of logic arises when we are told that all ova are formed by the time of birth. If this were so, why should there be an association between the occurrence

of Down's syndrome and the age of the mother; to put it another way, where does the extra chromosome come from if meiosis is already complete? One should not have to turn to other books to find out that protein "backbones" contain nitrogen as well as carbon, nor that the Hardy-Weinberg theorem only holds in the absence of selection. These are, however, minor blemishes and do little to detract from a fascinating book—a book of ideas, as the authors put it—which can be strongly recommended to biologist and non-biologist alike. Comparative physiologists may not think so highly of it, but you must read it to find out why.

Life: Cells, Organisms, Populations (Freeman: Reading, £10.20), edited by E. O. Wilson *et al.*, by contrast, is very definitely a textbook. It is an abbreviated version of the same authors' *Life on Earth* (reviewed in *Nature*, **247**, 582, 1974) and follows the same plan. There are five sections, entitled "The Cell", "Multicellular Life", "Origin and Diversity of Life", "Strategy of Evolution", and "Alternate Futures". It is clearly written, and although there are eight authors, is cohesive and not repetitive. An admirable feature of the book is the quality of the illustrations—there is a profusion of clear line drawings supplemented by good photographs where appropriate; but having said that, one might still ask why a section of a compound eye is not included as well as, or in place of, the surface view on p237.

A volume such as this may be used in two rather different ways; it may form the foundation of a course, as the authors suggest, or it may be used more as a work of reference. To base a course on a single text is probably unsound anyway, and although much of the content is relevant to advanced school studies, a substantial part would not normally be encountered until the first or second year as an undergraduate. It should thus find its rightful place alongside other such compendia in both schools and universities. As a work of reference, it is encouraging to see that the index contains about 2,400 entries, but even if you thought that you remembered seeing Gause's principle, Dollo's law or Gloger's rule in the text, you will not find them in the index; nor will you find "sympatric", except under speciation, and "allopatric" not at all.

The Science of Evolution (Collier Macmillan: London, £12), by W. D. Stansfield, is one of several books on evolution which have recently been published, and is perhaps most directly comparable with *Evolution* by Dobzhansky, Ayala, Stebbins and Valentine (reviewed in *Nature*, **270**, 457, 1977). Both are advanced texts and both cover much of the same ground, but whereas *Evolution* suffers from the not uncommon failings of multi-author books—some repetition and lack of integration—Stansfield's book provides a logical and well balanced pro-

gression. Conversely, the advantages of a multi-author volume are seen to good effect in *Evolution*, in which relatively specialised topics are developed in great detail. For these reasons, this latter book is to be recommended as a specialist text for use in seminars and tutorials, whereas the other book provides an excellent account of evolution for more general use. *The Science of Evolution* is divided into four parts: the framework of evolution, the mechanism of heredity, the forces of change, and the generation of taxa; the book is well illustrated.

When formulating his selection models, one cannot help thinking that unnecessary complication is introduced by treating the relative fitnesses differently in each of four models. It would surely be less confusing, especially to those who are not mathematically inclined, to apply the selection coefficients s and t consistently to the homozygous classes and choose values for them appropriate to the degree of dominance; in this way, generally applicable formulae for $\bar{W}$ and Δq may be obtained. Less excusable is the use of the correlation coefficient r to estimate heritability from the relationship between midparent value and that of their offspring (p204). Even if the heritability were 100%, a value of 1 for r would be obtained only if all offspring in a family were identical; it is the slope of the regression line which should have been used.

It is interesting (to a student of the debate that has continued over the past 50 years) that the evolution of dominance is discussed in some 100 pages, and in isolation, from the section on genetic homeostasis; perhaps in a future edition the two topics might be brought together. In the meantime, the relationship, or lack of it, could provide an informative tutorial discussion topic. Another fruitful idea might be revealed by cross-referral between sympatric speciation (p474) and the principle of competitive exclusion (p15); and if one extends the concept of a selection index (p205), it is hard to see the reasoning behind the unsubstantiated statement (p389) that overdominance "when operative at more than 100 loci, is likely to induce intolerable loads on the population".

The figures as a whole are excellent, but two deserve mention. Fig. 16.2 would give a much more satisfactory impression of a cline if the darkness of the shading correlated with the allele frequencies. The upper part of Fig. 12.31 illustrates an interesting pseudo-genetic event—the deletion of Borneo. Even though the pedigree of this map is not acknowledged, the deletion can be traced back several generations—it must be a neutral mutation.

E. R. Creed

E. R. Creed is Senior Lecturer in Genetics at University College, Cardiff, Wales.

Enzyme mechanics

Enzyme Structure and Mechanism. By Alan R. Fersht. Pp. 370. (Freeman: London, 1977.) Hardback £10; paperback £4.95.

IN the past twenty years there has been an enormous increase in the knowledge about the structure and mechanism of action of enzymes, but it is only in the past few years that a real understanding of how enzymes attain their huge catalytic rates is beginning to emerge. Perhaps it is this fact that is responsible for the plethora of books about enzymes that have been published recently; however, few books deal adequately with this topic at the student level. Dr Fersht was obviously aware of this problem and this book is his attempt—generally successful—to overcome this deficiency.

The book starts with a good general introduction to the three-dimensional structure of enzymes and some information on the X-ray diffraction methods used; but as the structure of enzymes is part of the fundamental background to this book one would have liked much more detail of the methodology involved. The author then proceeds to discuss the concepts of chemical catalysis, emphasising the importance of the transition state theory in the interpretation of reaction mechanisms and also the importance of general acid/base and covalent catalysis for enzymes. It is a pity that the part devoted to covalent catalysis was restricted to only a few of the structures found in enzymes, and to complete the picture something should have been said about flavins, biotin, folic acid and cyanocobalamin.

The weakest part of the book undoubtedly lies in the sections dealing

with the steady-state kinetics of enzymes. The author deals almost exclusively in terms of the equilibrium model and his representation of the reaction schemes (3.4, 3.10) are not only misleading but incorrect. The part that deals with multi-substrate systems is very scanty, and nothing is said about product inhibition. In view of the importance that the author attaches to the steady-state kinetic constants later in the book, it is surprising that he does not present better methods for abstracting these constants from experimental data. The chapter on practical kinetics is also unsatisfactory as initial rate is never defined and potential difficulties in its measurement never discussed. The weakness of this section makes me hesitate to recommend this book as a general text for senior undergraduates.

It is in the last part (chapters 9–12) that the author comes into his own and in which the greatest strength of the book lies. Here Dr Fersht deals with enzyme specificity and the theories of enzyme catalysis from the point of view of the transition state theory, and underlines the importance of entropy changes as the key to the large rate enhancements caused by enzymes. This treatment of a complex topic is lucid, comprehensive and amply illustrated with examples, although unnecessarily restricted to those enzymes the crystal structures of which have been solved.

In many ways this is an admirable book and one to be highly recommended, but a certain bitterness and lack of balance renders it less useful for the more general reader.

Bruno A. Orsi

Bruno A. Orsi is Senior Lecturer in Biochemistry at the University of Dublin, Ireland.

Enzyme kinetics

Steady-State Enzyme Kinetics. By Stanley Ainsworth. Pp. 255. (Macmillan: London, 1977.) £10.

A NUMBER of new books on enzyme kinetics have been published over the past few years, most of which have been reviewed in this journal (see *Nature*, **258**, 551, 1975; **259**, 255, 1976; **261**, 530, 1976; and **268**, 86, 1977); and a field which was once quite poorly served now has a wide choice of textbooks with different approaches. Because of this it would be hoped that any new book on this topic would make an original contribution either in its ability to explain concepts which many students find difficult or in providing new insights into the procedures of kinetic analysis. It is to this latter aspect that this book, which is intended for advanced undergraduate and graduate students, makes a particularly valuable contribution.

Except for a relatively brief introductory chapter on the nature of enzyme catalysis, the book is restricted to the treatment of steady-state and equilibrium kinetics with single and multi-substrate reactions, branched reaction mechanisms, isotope exchange methods and co-operativity being considered in detail. Unfortunately the treatment is somewhat uneven, and the sections on the effects of pH and temperature on enzyme activity are, for example, perhaps too brief to be adequate for the more advanced students. The derivation of the kinetic equation for the simplest single-substrate mechanism seems at first reading to be unnecessarily complicated, and I am afraid that many students might be put off the book at this stage, in favour of those providing more accessible treatments. The necessity for this approach, however, becomes apparent in those chapters in which the author develops his own methods for the presentation and analysis of kinetic mechanisms. It is in this area that the main



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strength of the book lies. The treatments that the author outlines, which are based on his own published work, offer advantages over those more commonly used and deserve serious consideration by anyone working in this area. Although the methods derived can usually be used for the interpretation of kinetic behaviour without recourse to the often tedious procedure of deriving full kinetic equations, the mathematical basis of this and other procedures are rigorously developed. Here again, the less numerate students could find the book hard going. The author is at his best in treating the mathematical basis of this topic, and although a chapter is devoted to the measurement of

initial rates and kinetics parameters, the treatment of practical details carries less conviction than the excellent sections on plotting methods and the concentration ranges that should be used.

This book has clearly been written by an expert in this field and it captures a rare sense of the author's enthusiasm. Although I do not believe that it can be recommended to any but the most able or committed undergraduates, it deserves to be widely read by more advanced workers for the many valuable insights that it provides.

K. F. Tipton

K. F. Tipton is Professor of Biochemistry at Trinity College, Dublin.

Physical chemistry and biological systems

Physical Chemistry with Applications to Biological Systems. By R. Chang. Pp. 538. Collier Macmillan: London, 1977.) Hardback £11.25; paperback £6.75.

"AND it's hard, and it's hard, ain't it hard, good Lord" is the quotation which prefaces chapter 1 of Professor Chang's book. The reader can be forgiven for perhaps thinking that this was a comment on physical chemistry by a student for whom this book was intended, namely one who is interested primarily in biological sciences. In fact the quotation is one from Woody Guthrie, but most teachers will recognise the general sentiment. For these students, physical chemistry often becomes of interest only when its relationship to biological sciences is recognised.

The title is a precise description of the contents. This is a text about physical chemistry to which biochemical applications have been added. Thus the whole of the topics normally taught in the first and second years of a chemistry degree course are covered. Indeed, the text covers a large section of structural information which is sometimes omitted from textbooks. The treatment is comprehensive and the information up-to-date. Throughout the text the emphasis has been successfully placed on understanding physical concepts. To this end, mathematical treatments and experimental detail have been deliberately excluded.

The biochemistry is added usually as a brief section at the end of each chapter, as, for example, in the case of biological oxidations and membrane potentials which appear in a total of four pages at the end of chapter 15 on electrochemistry. In addition, there are two chapters which scan photobiology and macromolecules. The biological content is therefore in no sense a comprehensive treatment, but is rather an indication of how physical chemistry can be useful in biology. In the relationship of biological applications to

physical chemistry, the book is similar in style to that by G. M. Barrow (*Physical Chemistry for the Life Sciences*, McGraw-Hill, 1974, £6.05). However, the biochemical content of Chang is considerably greater than that of Barrow and the book is far better produced.

If there is a real difficulty with Chang, then it is to decide for whom the book is really intended, as indicated by the selection of topics. Indeed, selection is the crux of the problem in writing a text which crosses the borders of conventionally recognised fields. For example, Barrow, in tailoring his classic text to the needs of biological science students, has felt it necessary or at least permissible to omit chemical bonding theory, statistical mechanics and phase equilibria. Chang, on the other hand, has left these topics in. Moreover, he has added topics such as liquid crystals, intermolecular interactions, spectroscopy, optical activity, photochemistry, photobiology and macromolecules. The purpose of these additions is to make the book also suit-

able "for a full-year physical chemistry course where a more rigorous text would be inappropriate". This it undoubtedly does. However, it is difficult to reconcile this aim with the original one of a text intended for premedical students. The book goes far beyond the needs of first-year medical and biology students, who are amply catered for at half the price by that excellent text *A Biologist's Physical Chemistry* by J. G. Morris (Arnold: London, 1974, £3.25). The latter covers just the basic core physical chemistry of immediate relevance to biologists with many biochemical applications and calculations throughout.

The place for a text as comprehensive as Chang's is either for full-time chemistry degree courses with options in biochemistry, of which there are an increasing number, or full-time biochemistry courses, such as that here at York, which have a strong initial grounding in chemistry and where the relevance of physical chemistry to biochemistry is emphasised at an early stage. Chang clearly breaks some new ground in attempting to cater for such courses. It is a pity that it does not contain a little more biochemistry, even if this were to be at the expense of the more advanced physical chemistry. Every effort has been taken to make things easier for the reader, even to the extent of using both c.g.s. and SI units simultaneously to assist with the transition from one set to the other. The book is excellently laid out, well illustrated, fully referenced (especially to biochemical topics) and very readable. At £6.75, it is within the reach of student pockets and deserves careful consideration.

G. L. Kellett

G. L. Kellett is Senior Lecturer in Biochemistry at the University of York, UK.

Assembly of biochemical knowledge

Biochemistry: The Chemical Reactions of Living Cells. By David E. Metzler. Pp. 1129. (Academic: New York and London, 1977.) \$26.95; £17.75.

THIS beautifully produced book is a magnificent assembly of biochemical knowledge. The amount of information presented considerably exceeds that of its nearest competitors, that is, the textbooks by Lehninger and by Stryer. In academic level the book would be more than adequate for a second-year university course in biochemistry. Some areas would probably provide a good reference text for final-year study. I feel, however, that the price will deter many student buyers. The book is well illustrated with very clear diagrams and photographs. Following each chapter there is a sizeable list of

references, to both standard reference books and to recent research papers. In addition, there are a number of study questions with each chapter. The author has adopted the use of boxes to set off specialist or ancillary information from the main flow of the text. The use of a cross-referencing system to group-related boxes scattered through the book is very successful.

By the author's own admission the theme of the book is more chemical than biological or physiological. Physical aspects are generally given an adequate treatment covering two chapters. These include sections on thermodynamics, electrode potentials, bonding, packing and cooperativity. Mention is made of many physical techniques, but I feel that some of the fundamentals, such as centrifugation and chromatography, are rather ignored.

Enzyme kinetics and enzyme mechanisms receive adequate treatment and there is a large and very complete chapter on coenzymes (88 pages). The reactions

and pathways of metabolism are clearly explained and extensively covered in four chapters (312 pages). Although there is some discussion of the regulation of metabolic processes, there is little mention of the interplay of different cell types and tissues to create an integrated metabolic whole in complex organisms. Considering the complexity of the field, biochemical genetics, nucleic acid and protein synthesis receive a very clear and up-to-date treatment.

Some areas receive more consideration than in most biochemistry textbooks. For example, there is an entire chapter on membranes and cell coats, a section on neurochemistry, a section on differentiation and developmental biology and a short appendix on the construction of molecular models.

Physical chemistry

RECENT generations of chemists have learned their physical chemistry from W. J. Moore's universally used text *Physical Chemistry* (Longman: London; Prentice-Hall: Englewood Cliffs, New Jersey), which replaced Glasstone and heralded a new era in the subject. Now after several editions there is a widespread feeling both amongst teachers and taught that a replacement is due; succeeding editions have passed their peak; the fourth UK edition was much preferred to the fifth.

For a large number of contemporary students, Moore's *Physical Chemistry* is too difficult, especially in its most recent appearance. One possible solution is to preface a meaty text with a more palatable introduction such as J. R. Amend's *Introductory Chemistry: Models and Basic Concepts* (Wiley: London, New York and Sydney, £12; \$20). A beautifully produced book; if anything the pill is too highly sugared. In Britain it would be suitable for schools, and even in the USA perhaps too lightweight for a course on chemical principles except for students who had been exposed to virtually no science previously.

Aiming directly at the large market of the mainstream university course are three new books, discussed here in this reviewer's (ascending) order of preference. Least attractive is R. M. Rosenberg's *Principles of Physical Chemistry* (Oxford University: Oxford and London, £15.75), which is just a rewritten version of earlier texts; the usual mixture in a new vessel. It is occasionally somewhat muddled and presents an old-fashioned feel so that it is unlikely to inherit the lucrative market.

V. Fried, H. F. Hamerka and U. Blukis adopt the direct title *Physical Chemistry* (Collier Macmillan: London, £12.75). This is altogether a more substantial work, although it may in places prove too difficult for many students. It is, however, very up to date. Defects are those

There is a rather novel chapter entitled "Light in Biology", which covers absorption of light by matter, spectroscopy, circular dichroism, fluorescence. This then leads on to photosynthesis (light and dark reactions), vision and bioluminescence. This particularly exemplifies one criticism I would make of the book—the chapters are too long (only 16 chapters for a text of 1,050 pages). To the student meeting the material for the first time the quantity and range of information in each chapter is likely to be quite daunting.

In conclusion, an excellent reference book in many ways, but one more likely to appeal to teachers than to students.

E. D. Saggerson

E. D. Saggerson is Lecturer in Biochemistry at University College, London, UK.

common to multi-author works, such as an inconsistency in the use of units; trivial but irritating to the student. In fact eight chapters of the book have appeared separately in 1975 as *Quantum Theory of the Chemical Bond* with H. F. Hamerka as the sole author, suggesting an amalgamation of separate sections rather than a fusion of minds on a single subject.

Physical Chemistry by P. W. Atkins (Oxford University: Oxford; Freeman: San Francisco, hardback £15; paperback £7.95) represents the biggest change in style from earlier works. The first glance shows diagrams which are not to be found in rival texts and the quality of the figures, together with the author's widely acknowledged facility to give qualitative interpretations of mathematics encourage a positive view. Not surprisingly the book is particularly strong on quantum mechanical aspects such as angular momentum. Major topics are covered twice, firstly in a chapter headed "Concepts" and followed by a separate chapter on "Machinery". This separation of the underlying physics from its ramifications should help students to achieve some essential understanding before being bogged down by the technicalities which may obscure the heart of the matter. The other innovation which may not be so universally welcomed is the very extensive use of worked examples in the main body of the text. These reduce the readability and chattiness which are a feature of the author's earlier book on molecular quantum mechanics and indeed of Moore's *Physical Chemistry*. Nonetheless, Atkins' book represents the strongest challenge yet made to the latter's supremacy.

Interestingly, the two more recommended next texts both choose the once-despised topic of electrochemistry for their final flourish, which is a fair statement of current importance, whereas Rosenberg only affords the topic some twenty pages.

Two more specialised text to appear include *Contemporary Quantum Chemistry* by J. Goodisman (Plenum: New York and

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London, \$39) and *Molecular Symmetry and Group Theory* by A. Vincent (Wiley: London and New York, £5.90; \$12). Goodman is a respected researcher who it seems, like many others, has taught a course and experienced the thrill of understanding this most satisfying topic. Like many others he has felt compelled to write up his version of what was obviously an excellent course. There is, however, very little justification for the inclusion of the word *Contemporary* in the title. It is just the old, still-exciting story of helium and the hydrogen molecule as already available in scores of texts: a good book but only a new growth from old seed, not a mutation we have not seen before.

Vincent too seems to have been stimulated by giving a successful course. His book, subtitled *A Programmed Intro-*

duction to Chemical Applications, aims at teaching undergraduates to use group theoretical results rather than to understand them. It is hard to see other teachers adopting another's ideosyncratic detailed course but as a student self-teaching aid it will be welcomed by those whose group-theoretical requirements are restricted to the use of character tables.

All the books reviewed here reflect the strength of contemporary physical chemistry at its physics boundary but the other frontiers of this linking subject, such as those with organic chemistry or biophysics, are currently under-emphasised.

Graham Richards

Graham Richards is Lecturer in the Physical Chemistry Laboratory, University of Oxford, UK.

Organic chemistry

FAT American organic textbooks commonly fall between two stools. They cover more ground than we need for a first-year university course in Britain but they cover it too thinly for second- or third-year courses. Who, for example, learns to interpret nuclear magnetic resonance (NMR) spectra from a general text? Yet three books published recently give some space to NMR spectra along with assorted fragments of natural product chemistry: *Basic Principles of Organic Chemistry*, by J. D. Roberts and Marjorie C. Caserio (W. A. Benjamin/Addison-Wesley: Menlo Park, California, \$24.95); *Organic Chemistry*, by D. C. Neckers and M. P. Doyle (Wiley: London, New York and Sydney, £16.50; \$27.90); and *Principles of Organic Chemistry*, by T. A. Geissman (Freeman: Reading, fourth edition, £14.60). Admittedly, Roberts and Caserio do the job well, and Neckers and Doyle moderately, but none of the three approaches the familiar Tedder and Nechvatal, *Basic Organic Chemistry* (Wiley: London, five volumes, 1966-76), in its thorough treatment of advanced topics, or Streitwieser and Heathcock's *Introduction to Organic Chemistry* (Macmillan, 1976) in its thorough treatment of first-year material.

A fourth book, *Advanced Organic Chemistry* by F. A. Carey and R. J. Sundberg (Plenum: New York; \$47.40 each part) is quite different. Rightly, for an advanced text, it assumes the basic knowledge of general chemistry which most first-year university students have in Britain and embarks on a two-volume text which is simply a triumph. In some ways, it is the successor to Gould that we have been waiting for, but it is Gould's approach transformed, tightened up, and given the modern emphasis on synthesis. I'm sure it will prove excellent for second- and third-year undergraduates, graduates, and lecturers preparing courses.

Part A, *Structure and Mechanism*, introduces both the valence bond and molecular orbital approaches, does stereochemistry and conformational analysis properly with excellent diagrams, and gives an introduction to methods of investigating reaction mechanisms including isotope effects, Hammett and Bronsted plots, and solvent effects. The rest of part A deals with reaction types—substitution, elimination, addition and the like—assuming a knowledge of physical methods, particularly NMR. Good features are the frequent tables of facts: typical reactions, pK_a values, stereochemical consequences, reaction rates, and so on, all with references to the original literature. The principles and methods introduced in the first few chapters are applied to each reaction type so that the student's knowledge is reinforced by immediate and repeated practical application. The synthetic applications of each reaction type are given briefly at this stage. There is a major and satisfying treatment of pericyclic reactions. Each chapter ends with challenging problems and literature references to the answers are given. One of the aims of the authors is to turn the student to the literature, and the many references in the text, tables, and problems should do the trick.

Part B, *Reactions and Synthesis*, is a third turn of the reinforcement spiral. The first chapters introduce wholly new material in the major carbon-carbon bond-forming reactions. Then follow chapters dealing with the synthetic applications of reaction types met in part A but differently classified under reaction type (cycloadditions, oxidations, aromatic substitutions) or compound type (organometallic, carbenes, and so on). The examples are of course different from those in part A and are chosen for their synthetic merit. Finally, we have chapters on multistep and macrocycle synthesis.

My only criticism would be that there is no earlier treatment of strategy such as the one in the excellent course book from

the Open University, *Principles of Organic Synthesis*, Second Level Science Courses Book S24-9. Carey and Sundberg is one of the major books of the decade and I strongly recommend all teachers of organic chemistry to buy at least the paperback at £7.90 for each part.

Neckers and Doyle is the only wholly new book of the other three. Paradoxically it has a quaint old-fashioned air about it. Fischer projections are used to explain stereochemistry; chiral centres are called asymmetric centres; there are tedious problems demanding 20 or 30 parrot definitions at the end of each chapter; IR spectra are linear in microns; and most amazingly there are almost no curly arrows even in chapters on mechanism! Some students will find this a useful first-year university text as there are good solid elementary treatments of how reactions occur and synthesis, and the reinforcement method is used to advantage. The approach is a mixture of reaction type and compound type.

Roberts and Caserio is a much better book with a refreshingly realistic approach. Molecular orbital and valence bond treatments are reconciled, much is made of the practical side of organic chemistry, and there is a fair bit on the industrial side too—a whole chapter on polymers. With chapters on pericyclic reactions and transition metal compounds this book is advanced enough to take most students to the end of their second year. There is a bit on natural products, a chapter on amino acids and peptides, and a quick flash of nucleic acids. The approach again mixes reaction and compound type and does so quite sensibly. This would be a good book for a student who has done little or no organic chemistry and wants to be taken slowly through to more advanced stuff. Prostaglandins are discovered on p1492.

Geissman has now reached its fourth edition and has a rather tired air. Structure is dealt with by valence bond and resonance (this is apparently a new theory replacing the old tautomeric effect!). Formulae are poor—we have square alkanes and alkanes and bent alkynes, conformational drawings with strange bent axial bonds, and most reactions drawn without mechanism. The several "most suitable" representations of chiral molecules introduced in the stereochemistry chapter include neither the one commonly used by organic chemists nor the odd one Geissman himself uses in later chapters. There are curious bits of exotica like the senecio alkaloids, two whole chapters on carbohydrates, but no mention of pericyclic reactions, not even a simple Diels-Alder reaction, surely one of the most important of all organic reactions. I cannot recommend this book.

Stuart Warren

Stuart Warren is Lecturer in Organic Chemistry at the University of Cambridge, UK.

Principles and applications of isotope geology

Principles of Isotope Geology. By Gunter Faure. Pp. xii+464. (Wiley: London, Sydney, New York and Toronto, 1977.) £13.60.

THE late 1940s and early 1950s saw the birth of many ideas and techniques which in the intervening years have grown into the subject of isotope geology. It is a measure of the way in which the subject has developed that this introduction to the principles and applications of isotope geology contains 464 pages, 21 chapters, 2 appendices and a host of literature references. Although the practitioners of isotope geology still make up a relatively small but growing proportion of Earth scientists, the implications of isotope measurements find their way into such a wide variety of geological problems that it is essential for every geologist to have a good awareness of the methods and principles involved if he is to appreciate the power as well as the limitations of the isotopic information to which he may have access.

Dr Faure's book is aimed particularly at the senior Earth sciences undergraduate student entering the field of isotope geology for the first time. I have a feeling also that most practicing geologists would find this a very useful addition to their bookshelves. It is not only a book that can be read systematically from cover to cover but one that can be referred to from time to time when the need arises to refresh the memory as to how some particular isotopic tool 'works' or as to what are the assumptions necessary to draw useful conclusions.

The book is set out in three parts. Chapters 1-5 are introductory, covering historical aspects, atomic structure, principles of radioactive decay, and mass spectrometry (the basic tool of isotope work). The meat of the book is in the remaining 16 chapters. The many dating methods now in use are described in chapters 6-17, along with examples (from the literature) of their application. The reader meeting the subject for the first time will soon realise that the geochronologist now expects to answer much more than the simple question "How old is this rock?", whatever that means. Rather, he expects, in suitable circumstances, to be able to unravel an often complex history of events, to provide constraints on the rock's origin and mode of formation, and to provide answers to more general questions such as the origin and evolution of the continents and the evolutionary history of the upper mantle.

The last four chapters cover the field of stable isotope studies (hydrogen, oxygen,

carbon and sulphur). The applications are too many to summarise in a short review but range over topics as diverse as snow and ice stratigraphy, palaeothermometry, and hydrothermal ore deposits. The author makes no strong demands on the mathematical skills of his readers, and students in particular may find the calculations set out in the text and the many examples following each chapter useful.

This is a very comprehensive book and no doubt specialists in any particular branch of isotope geology may find small points or oversimplifications with which they disagree. For example, the statement

on p167 that interference corrections limit the use of ^{40}Ar - ^{39}Ar dating to samples older than 1 Myr is incorrect. The large field of extraterrestrial isotope studies is hardly mentioned except where it impinges fairly directly on terrestrial applications. However, such criticisms are minor, and this well thought out and well executed introduction to the principles and application of isotope geology should be of use to students and practicing Earth scientists alike.

Grenville Turner

Grenville Turner is Senior Lecturer in Physics at the University of Sheffield, UK.

Physicochemical principles and geological systems

Chemical Petrology. (With Applications to the Terrestrial Planets and Meteorites.) By R. F. Mueller. Pp. 394. (Springer: New York, Heidelberg and Berlin, 1977.) DM 72.80; \$32.10.

CONSIDERABLE advances have been made recently in the application of physicochemical principles to geological systems. This renewal of interest in chemical petrology has stimulated a demand for course texts which both summarise and explain the techniques used and several have been published in the past year. Mueller and Saxena's book is an ambitious attempt to treat the origin and evolution of igneous and metamorphic rocks, related fluids, the terrestrial planets and meteorites from the common viewpoint of the underlying principles of physical chemistry. The book's success in condensing much relevant information will be readily apparent to the reader. As a teaching medium, however, it suffers from its wide scope which results in subjects frequently being dropped before being discussed to a satisfying depth.

The book may be roughly divided into four parts: "Review of Thermodynamics and Kinetics"; "Meteorites and Planetary"; "Metamorphic Petrology"; and "Igneous Petrology". The first part is a review of many of the equations used, including a treatment of the effects of gravity on chemical potential. I found the presentation dry. For the reader without a good working knowledge of thermodynamics (for example, a knowledge of Maxwell's equations), many relationships do not follow obviously, and this may prove a deterrent to many with backgrounds in the Earth Sciences.

Three chapters are devoted to petrogenetics in Space, meteorites, and petrology of the terrestrial planets. These are interesting and informative, and the dis-

cussion of condensation reactions is particularly timely. It is regrettable, perhaps, that lunar petrology receives such a limited treatment in comparison with the space allotted to Venus.

The treatment of metamorphism continues the physicochemical paradigm of the book by discussing the origin of microtextures from a thermodynamic viewpoint, and the kinetics of crystal grain growth and of metamorphic reactions. This is followed by a review of experimental studies of the P-T stability of various pure mineral assemblages followed by a rather brief analysis of the effects of solid solution. A chapter is devoted to metamorphic mineral facies followed by further discussion of experimental studies of metamorphic systems and a rather disappointing discussion of Schreinemaker's rules.

Igneous petrology is introduced by considering some properties of silicate melts (water solubility density, viscosity, immiscibility). The differentiation of magmas is considered first by analysing diffusion rates in melts and the separation of a fluid phase before discussing the role of crystallisation with a limited use of phase diagrams and variation diagrams. For a book concerned with the physicochemical approach to petrology, it is most surprising that the thermodynamic approach of Carmichael and his colleagues is not mentioned.

This is an interesting, different and stimulating book. Its greatest inadequacy lies in the overemphasis of principles with too few examples and an almost complete lack of worked numerical problems. It is difficult to see its role as a course text especially since the price is likely to prevent most students from purchasing their own copies. It is well produced, although the editing might have been more careful, with some authors' names misspelt throughout the text but correctly in the index and the occasional typographic slip in equations. I am glad, however, to have a copy on my shelf.

D. G. Fraser

D. G. Fraser is Lecturer in Geochemistry at the University of Oxford, UK.

Tropical climatology

Tropical Climatology: An Introduction to the Climates of the Low Latitudes. By S. Nieuwolt. Pp. 207. (Wiley: London, Sydney, New York and Toronto, 1977.) £6.75; \$15.

Tropical Climatology is primarily intended for students studying geography at Universities within the tropics, though it contains much information useful to students working elsewhere. The level is about that of a first-year geography course in a British University. The book assumes that the reader has some basic knowledge of general climatology, but only a limited knowledge of mathematics and physics. According to the author, the book takes a geographical viewpoint, which concentrates on the results of the physical processes in the atmosphere, and their importance to mankind, rather than on the processes themselves. The book covers most aspects of tropical climatology, including the general circulation of the tropical atmosphere, tropical disturbances, precipitation and applied tropical climatology.

Nieuwolt starts his book with a discussion on the term "tropics" and the distinguishing features of "tropical climates". In the early chapters on radiation and temperature, he continues to stress the general uniformity within the deep tropics. These chapters would have been improved by the introduction of a discussion of the energy balance of vegetated and dry surfaces, as the tropics are not uniformly wet. Evaporation limits the temperature over wet surfaces to 32 °C or below, whereas over dry surfaces it can rise to much higher values.

Nieuwolt devotes the last half of his book largely to aspects of water in the tropical atmosphere. He starts with a brief discussion of evapotranspiration, humidity, condensation and clouds. The principles of evapotranspiration are explained in clear simple terms. The nature of tropical clouds are discussed, together with their relationship to precipitation patterns. Tropical rainfall is discussed, together with theories of its formation.

Nieuwolt ends his book with a chapter on applied tropical climatology. Here he suggests that one field in which tropical climates can make significant contributions to the world's economy is in the generation of power. Thus, the high amounts of rainfall which are regularly received in many elevated areas of the tropics, constitute a reliable basis for the construction of hydroelectric power stations. The author claims that about 55% of the world's total potential of this form of energy is located in the tropics. Solar energy is another source of power in which the tropics are potentially rich,

particularly where the seasonal variations in actual hours of sunshine are relatively small. Nevertheless, Nieuwolt states that the main function of the tropical climates as a natural resource is in relation to agriculture, and he considers both solar radiation and rainfall in relation to tropical crop production.

The book as a whole is easy to read, well produced, and forms an excellent introduction to the climatology of the tropics. It should be ideal for University geography students studying within the tropics and in particular those living in

Africa. Temperate latitude students will find that their needs are better served by such books as *Introduction to the Atmosphere* by H. Riehl (McGraw-Hill, 1965) and *Atmosphere, Weather and Climate* by R. G. Barry and R. J. Chorley (Methuen, third edition, 1976). Even so, they will find it a useful book for additional reading on introductory courses.

J. G. Lockwood

J. G. Lockwood is Senior Lecturer in the School of Geography at the University of Leeds, UK.

Oceanographic sciences

MODERN BOOKS, like modern museums, seem able to display their exemplary contents in attractive, eye-catching ways. Four recently published books on the oceanographic sciences are well made and well illustrated; their style is comparable but they vary in their aims and in the extent to which they succeed in achieving them.

To me the most visually attractive is that of J. G. Harvey: *Atmosphere and Ocean: Our Fluid Environments* (Artemis: Horsham, Sussex, UK, £3.75). Although the cheapest of the four it has good paper, clear print and is well illustrated. It also seems to me the least presumptuous—writing for those with no previous knowledge of meteorology and oceanography and with little physics or mathematics, the author states results rather than proving them, with enough supporting argument to make them seem reasonable. To give an example of his scope: group velocity is mentioned; meso-scale oceanic eddies and double diffusive convection not. I share the author's view that there are benefits to be gained from studying the atmosphere and the ocean together: his book is a useful introduction for non-specialists.

The Ocean-Atmosphere System (Longman: London, £5.50) by A. H. Perry and J. M. Walker, sounds, and is, more portentous. The authors have taken on a harder task, seeking to "treat meteorology, climatology and physical oceanography" for more advanced students in "the broad fields of geography, geophysics, environmental science, marine biology and nautical studies". It seems to me doubtful that such an aim can be achieved: specialised branches of fluid mechanics surely need a good grounding in the the fundamentals? To me their treatment is too historico-geographical, too prone to quote detail rather than basic principle, too broad and too shallow. Again as an example: group velocity is not mentioned, though there are ritual references to Lamb's treatise and Phillips'

monograph. As well as the extensive references, there are explanatory notes to each chapter: "Isotropic turbulence is determined solely by the nature of the surface over which the wind blows . . ." is an (I hope atypical) example. The classic temperature-salinity diagram is briefly (and incompletely) described in the appendix. The book is an attempt to fill a gap in the literature but the gaps seems likely to continue to exist, as it is between differently trained people.

F. P. Shepard, the author of *Geological Oceanography: Evolution of Coasts, Continental Margins and the Deep-Sea Floor* (Crane, Russack: New York, \$10.50), is an emeritus professor at the Scripps Institution of Oceanography who was a pioneer in the subject and has been contributing to it for over 50 years. His book is intended for beginning students and for the general public—it is descriptive, with lots of charts, diagrams and photographs but no formulae. The chapter on waves and currents is less authoritative than the others: no group velocity, and a bit shaky on wave velocity; but it has useful advice for surfers and for swimmers in rip currents or on a coral reef. The marine geology is confident and clear, if perhaps a bit discursive. Redolent of the Pacific, rather than the Atlantic or Mediterranean, I was sorry not to see some reference to side-scan sonar or to modern magnetic methods. Professor Shepard has written an interesting introduction rather than a textbook.

Oceanography: A View of the Earth (Prentice-Hall: Englewood Cliffs, New Jersey, £13.55) by M. Grant Cross, is on a bigger scale than the others: more than twice the pages, more than twice the cost. It is the second edition (first in 1972) of his attempt to survey oceanography (marine physics, marine geology and geophysics, marine biology, a little marine chemistry) for beginning students or for those who need only a nodding acquaintance with the science of the sea. In this he has to a great extent succeeded: as examples, he gives a good description of group velocity (though not using it in a later section) but regards sonar as a method for the sonic detection of other vessels or objects and for underwater

signalling, not referring to it as a tool in marine geology. The basic difficulty of preparing elementary book about scientific subjects is illustrated by the first of his five useful appendices, which starts by describing how large and small numbers can conveniently be written as powers of ten and giving rules for multiplying and dividing them. Dr Gross seems to have overcome the difficulties as well as anybody could, producing a balanced introduction which will be useful to many

non-specialists. Perhaps in a third edition he will correct the few remaining errors, including the statement that the longest waves in the Pacific are 34 m long and those in other oceans 15 m. Of course, he meant wave heights but even then the statement is surely overdefinite and quite probably false.

H. Charnock

H. Charnock is Professor of Physical Oceanography at the University of Southampton, UK.

Geomorphological basics

THE appearance of yet another general text on geomorphology raises several questions: is there a need for it? Does it compare favourably with its competitors? Is it good value for money? The answer to all these is undoubtedly yes. R. J. Rice has built on his experience in teaching geomorphology to first-year undergraduate geographers at the University of Leicester to produce a book that aims "to build on the foundations normally provided by sixth form studies at school". *Fundamentals of Geomorphology* (Longman: Harlow, UK, £6.50) is therefore chiefly written with the British student in mind, to form a bridge between school and more advanced university studies in geomorphology.

Unhappily, there exist few texts that adequately review the subject at this level and combine an up-to-date treatment with a concise and readable style. The past 10 years have seen the appearance of at least seven or eight general geomorphologies, even if one excludes American undergraduate texts, translations and second editions, yet the problem of what to recommend to one's first-year students in geomorphology has remained. A. L. Bloom's *The Surface of the Earth* (Prentice-Hall, 1969) is admirably readable but, at 152 pages, too slight; A. F. Pitty's *Introduction to Geomorphology* (Methuen, 1971) presents a full treatment but suffers from a complex layout and the inclusion of some material more appropriate for second-year students; K. W. Butzer's *Geomorphology from the Earth* (Harper and Row, 1976) is too expensive; R. J. Chorley and B. A. Kennedy's *Physical Geography: A Systems Approach* (Prentice-Hall, 1971) offers a new approach but one that many first-year students find difficulty in understanding; and so one could go on. Moreover, not one of these texts offers anything more than a perfunctory treatment of endogenous processes, and many omit this important field entirely.

Rice devotes one-quarter of the book to the Earth's first-order relief features and structures and to global tectonics. It is worth commenting that even this proportion is small compared with the

attention given to this topic by geomorphological texts published in the Soviet Union, where great importance is attached to morphostructures and morphotectonics in understanding the Earth's relief. Rice describes his approach as "quantitative but non-mathematical". Numerical data and analysis are not lacking; relationships such as the Bernoulli equation for the energy of a stream and Glen's flow law for glacier ice are introduced as appropriate, but always in a clearly explained manner.

The book contains seventeen substantial Chapters, and there is no space here to set out the contents in detail. As already mentioned, Part I (five chapters) is basically concerned with the application of geophysics to an understanding of the primary relief features. Plates and plate tectonics figure largely in this, but there is also discussion of localised crustal movements and of geochronology (with particular emphasis on the Cenozoic). Part II takes the student through various aspects of subaerial denudation: weathering, slope description and measurement, mass movements, stream flow and sediment transport, drainage basin analysis, and models of landform evolution from Davis to Hack. It is a pity, however, that there is very little mention of aeolian processes in arid environments, and deserts in general receive only minor attention.

Part III covers glacial and periglacial phenomena (four chapters); Part IV looks at coastal processes, sea-level change and the evolution of coastal forms (three chapters). The arrangement is logical and leads on easily from work of advanced level school standard. There are clear sub-headings and it is easy to find one's way around, even without recourse to the list of contents or the index. The style of the maps and diagrams does not greatly appeal to this reviewer: there tends to be too much use of thick lines and dark shading, but the selection of illustrations is well judged. Instead of a massive reference list at the end of the book, selected references and recommendations for further reading are set out at the end of each chapter.

Although the geomorphological specialist will find many cases of oversimplification in presentation and points to disagree with, the book succeeds well in compressing a broad field of study into a limited space, conveying some idea of

the directions of modern research in the subject and stimulating the student to further reading. It fits the market for which it is designed admirably, and its cost compares favourably with competing texts.

Landscape Processes: An Introduction to Geomorphology (Allen and Unwin: London, £2.50), by D. & V. Weyman, is a slim volume designed to cater for the needs of sixth-formers, and aims to introduce them to some of the important concepts of modern geomorphology. Processes rather than landforms are emphasised, and the reader is shown how measurement of process has assisted the development of geomorphological theory. Most attention is paid to humid environments: nearly one-third of the book overall is concerned with the movement of water in river or ground-water systems. On the other hand, endogenous processes and volcanic activity are dismissed in one page, "because these topics are dealt with at length in many other books". This is not a very convincing excuse, for there is a notable lack of concise up-to-date accounts of these topics at this level of treatment, and it is a pity if the sixth-former goes out with the idea that geomorphology is solely concerned with exogenous processes.

The arrangement of the book is clear and logical. After a brief introductory chapter, there are sections dealing with "Humid Landscapes" (37 pages), "Arid and Semi-Arid Landscapes" (8 pages), "Glacial and Periglacial Landscapes" (13 pages), "Coastal Landscapes" (10 pages), and "Landscapes of the Past" (10 pages). Illustrations are plentiful and attractively produced, and there is a short list of recommended further reading at the end, though this consists mostly of relatively advanced texts.

The style of writing is, on the whole, reasonably clear, but there are exceptions. Assuming that the book is being used by a student who has just been through an ordinary level course, the explanations of some ideas, such as the development of convexo-concave slopes (p32), of the long-profiles of rivers (p35), and the rotational movement of cirque glaciers (p64) may prove difficult to grasp. Indeed, there might be something to be said for using this book in conjunction with, or subsequent to, the reading of some more traditional textbook of geo-

morphology, such as B. W. Sparks' *Geomorphology* (Longman, 1960), M. J. Selby's *The Surface of the Earth*, Volume I (Cassell, 1967) or A. L. Bloom's *The Surface of the Earth* (Prentice-Hall, 1969).

The book is well produced, with few errors, but a little expensive compared with some other recent texts.

Clifford Embleton

Clifford Embleton is Reader in Geography at King's College, University of London, UK.

New texts in electromagnetism

I THOROUGHLY enjoyed reading J. Crangle's *The Magnetic Properties of Solids* (Arnold: London, hardback £10; paperback £4.95). It is written in an easy confident style which appealed to me and left me feeling that my understanding of the subject had been decidedly improved. The appearance of the book is reasonably pleasing too, with clear mathematical layout and good diagram locations. The unregistered right margin is only a minor irritant and has presumably helped to keep the price down.

This book is one of a series edited by Professor Bryan R. Coles under the general title *The Structures and Properties of Solids*. It is aimed at the "senior undergraduate studying physics in a British university" and to a lesser extent at other undergraduates, postgraduates and practising scientists. The level seems to me to be absolutely right for the specialist or optional courses which are often offered in the final year after a basic solid-state physics course. There are generous numbers of diagrams, many displaying experimental data (not always referenced—for example, figures 3.7, 4.13 and 6.13) which support the text. There are no problem sets, although this is perhaps not a serious defect. The heart of the book is, I suppose, the central group of five chapters: "Localized Magnetism Associated with the Ion Cores", "Magnetism Associated with Band Electrons", "Antiferromagnetism, Ferrimagnetism and Helimagnetism", "Magnetic Interactions and Hyperfine Fields", and "Domain Magnetism". There is also a helpful introductory chapter on basics and units (the SI system is used consistently, with c.g.s. conversions also given in brackets) and two sensible final chapters, one on experimental techniques (extremely valuable) and the other on practical applications.

I have to report that I was completely out of sympathy with the second book, R. C. Cross's *Electricity and Magnetism* (Longman: Sidney and Harlow, UK, paperback £3). Again it is one of a series, under the editorship of J. L. A. Francey, and it was written "for students taking a first course in physics at university or college". Its level seems to me to hover unhappily between an advanced level at school and that of a proper electromagnetism course for undergraduates (at a British university). In my experience of lecturing on electromagnetic theory to first-year undergraduates, students become restive if they suspect that their schoolwork is being wheeled out again. It can be done briefly, but then no textbook is needed. So I am puzzled about Cross's intentions. It is clear from what he says and from internal evidence that the book is not

intended to be a physics degree-level course. To give an example, the treatment of electromagnetic waves, though interesting and physical, is rather brief and avoids heavy vector manipulation by not presenting the wave equation at all. This in my view disqualifies the book as a main recommendation for a full electromagnetism course. But then are there any universities who can spare the time for two full courses on electromagnetism, one at the level of this book and a second at degree level?

I also found as I read the book a number of detailed points which made me unhappy right from the beginning. In the hydrogen atom, the electron does *not* spend most of its time moving around the surface of a sphere of radius 5.3×10^{-11} m (p7). $F = q_1 q_2 / 4\pi\epsilon_0 r^2$ does *not* give the force between charged objects (a common student fallacy) unless those objects are small compared with their separation (p21). The argument (on p36) that electric field lines outside a conductor must be perpendicular to the surface because

otherwise electrons would move inside the conductor, is not convincing without more discussion. An electric kettle operated at 0.045 W would never boil (p72) although admittedly for thermal rather than electrical reasons. On p156 it is remarked that students are often bothered by the fact that there may be electric fields, at points where $B=0$, which are induced by fluxes changing elsewhere. Those students are unlikely to be helped by the bare statement that "this is indeed a remarkable feat of nature" coupled to a rather doubtful analogy.

The book is pleasantly laid out and reasonably priced. It contains quite a large number of problems, generally helpful diagrams, and basic equations (not numbered, presumably deliberately) which are as light as possible on calculus and vectors.

D. S. Betts

D. S. Betts is Lecturer in Experimental Physics at the University of Sussex, UK.

Electrical properties of metals

Electrical Properties of Metals and Alloys. By J. S. Dugdale. Pp. 292. (Edward Arnold: London, 1977.) Hardback £10.50; paperback £4.95.

APART from their mechanical properties, the electrical properties of metals are surely those which are most widely exploited—and what wonderful properties they are! The electrical conductivity, the Hall effect, thermoelectricity—familiarity should not blind us to the remarkable nature of these phenomena. Their widespread applications as well as their intrinsic interest demand a reasonable degree of understanding by any physical scientist. Unfortunately, once one ventures beyond the classical Drude treatment, the theory of electrons in metals can become very complicated, and the physics which underlies electrical conductivity and its associated properties can become lost in an assembly of matrix elements.

Professor Dugdale does not lose sight of the physics in the present carefully written volume. He takes great care to explain the physical ideas that underlie the various phenomena, not only in the introductory chapters but also in the detailed exposition which follows. He uses simple arguments with the minimum of mathematical sophistication to explain the dynamics of electrons in metals and the various scattering mechanisms to which they are prone. The Boltzmann equation, scattering from static imperfections and phonons, the Born approxi-

mations, phase shift analysis and the Friedel sum rule, are dealt with gently but firmly.

In later sections more complicated phenomena are discussed. The Kondo effect, always a tricky item, is particularly well explained and there are good sections on phonon drag and Matthiessen's rule. The final chapters cover the electrical properties of the transition metals and of alloys. The level should not cause much difficulty to any reasonably competent second-year undergraduate, although it is unlikely that any undergraduate would wish to study in such detail all the topics covered.

It is inevitable that a reliance on fairly simple mathematics necessitates more wordy explanations and some readers, particularly those with a theoretical bent, might find the pace a little slow, but this is a small price to pay for the clarity of explanation which is evident throughout the book. It is certainly one to be recommended as supplementary reading for any introductory solid-state physics course.

This book is one of a series, *Structure and Properties of Solids*, and in my experience the quality of individual volumes in a series can be very variable. In the present instance this is not so. Each of the volumes I have read has been of a very high standard and the editor of the series, Professor Bryan Coles, is to be commended on his selection of authors and on the control which he has apparently been able to maintain over them.

H. M. Rosenberg

H. M. Rosenberg is Reader in Physics at the Clarendon Laboratory, University of Oxford, UK.

Electromagnetic radiation

Electromagnetic Vibrations, Waves, and Radiation. By G. Bekefi and A. H. Barrett. Pp. 664. (MIT Press: Cambridge, Massachusetts and London, 1977.) \$17.50.

THIS is a textbook for second- or third-year physics and engineering students who have already had courses in mechanics and in electricity and magnetism. The first two chapters go thoroughly over the groundwork of oscillations and waves with lots of examples, not necessarily from electromagnetism — pendulums, coupled springs, seismometers and sound waves. Maxwell's equations are briefly introduced, and used to deduce the wave equation; and then the book settles down to its main task of describing the behaviour of electromagnetic radiation. The aim is to provide a mathematically unsophisticated treatment of the subject, stressing modern applications. The authors succeed rather well in giving quantitative though not formal descriptions of a very wide range of phenomena. Perhaps almost too wide a range: the chapter on sources of radiation discusses not only accelerating charges, Hertzian dipoles and antennas, but also cyclotron and synchrotron radiation, *bremstrahlung*

and black body radiation. The synchrotron radiation from a highly relativistic beam is treated in a very back-of-the-envelope fashion, but interestingly, and with references for the student to follow up.

Photons are mentioned only once, when it is shown that the same expression for the radiation pressure can be obtained in the classical and quantum-mechanical pictures. For most of the topics treated—reflection, interference, waveguides, and so on—no problems are created by sticking to a rigorously classical approach. But a determination to explain everything in terms of oscillations does not work so well in introducing lasers and masers. Stimulated emission is a “process in which atoms jiggling in the correct phase relationship with the wave can give up some of their energy to the wave”. The only example of what is mysteriously called “preparing the medium” is with a maser operating with relativistic electrons, which is rather difficult to understand. There is no mention of the simple idea of optical pumping.

Most of the examples are by contrast clearly explained, and I think many students would find this book a good introduction to new ideas. **I. S. Grant**

I. S. Grant is Senior Lecturer in Physics at the University of Manchester, UK.

Probability theory

Elementary Probability Theory. By M. Hausner. Pp. 610. (Plenum: New York, 1977.) \$10.75.

THERE have been many very good books on probability theory published in recent years, ranging from the popular introduction to the comprehensive treatise. To be competitive in this field at any level, a new book requires a distinctive approach to the subject and a careful consideration of the intended market. The book under review is at a first-year undergraduate level, adopts a rather conventional approach and is likely to appeal only to a student who proposes to specialise in a study of statistics. This limitation is a pity because the book has the potential to fill a gap which exists for the science student who requires an elementary understanding of probability concepts as they arise in his particular subject, but presented and proven with a certain mathematical rigour. It fails to satisfy in this respect, not by the choice of material, which is admirably suited to the purpose, but by the absence of suitable illustrative examples. The numerous worked problems and more than four hundred exercises are concerned, almost exclusively, with dice, playing cards and other games of chance. The value of probability concepts for the scientist are totally overlooked.

Despite these reservations, the author

is to be praised for a splendidly lucid presentation of his subject enhanced by a thoughtful layout of tables, diagrams and formulae. Ideas of probability are developed from first principles, assuming no previous knowledge of the subject and requiring a very basic mathematical background of the reader.

Nevertheless, progress is rapid, from the fundamental concepts of permutations, combinations and elementary set theory, which are established in the first two chapters, to a consideration of the general theory of finite probability spaces. An interesting chapter on miscellaneous topics touches on random walk problems, which would have been an interesting area to explore in a scientific context. The treatment of random variables and the significance of variance is conventional, stimulated by a brief excursion into the theory of games. It is, perhaps, curious that a formal discussion of the Normal, Binomial and Poisson distributions is deferred to the final chapter and that no reference is made to the fact that many of the earlier problems could be solved by a consideration of the general properties of these distributions.

The book provides a very good basis for the comprehension and interpretation of statistics and can be recommended for the student who intends to proceed to this subject. **G. J. Lush**

G. J. Lush is Lecturer in the Department of Physics and Astronomy at University College, London, UK.

Group theory

Symmetry and Quantum Systems. By A. B. Wolbarst. Pp. 249. (Van Nostrand, Reinhold: New York, Toronto, London and Melbourne, 1977.) Hardback £9.95; paperback £3.95.

THIS is a pleasantly written and very readable introduction to the applications of group theory in quantum mechanics. The author assumes previous knowledge of physics and mathematics, which a British honours physics student would have in his final year. But it seems unlikely that room could be found in most final-year physics courses for a section on group theory substantial enough to justify using this book. It is, however, likely to be used more in the first stages of post-graduate study.

Wolbarst's book is comparable in standard to the books by McWeeny, by Joshi, or by Petrashen and Trifonov, but the approach is very different. The first third of the text is devoted to establishing a motivation towards the use of group methods in quantum physics. In this process, the author clarifies—in a way that should be helpful to all research physicists in training—the differences between real space and function space, between operators and their representations, and between transformations in real space and the corresponding operations in function space.

The next three chapters treat respectively representations of operators; representations of groups of operators; and irreducible representations. This mainly mathematical section is followed by four chapters in which physical applications are again to the fore—the relationship of symmetry in Hilbert space to symmetry in real space; the uses of continuous groups; perturbations; and symmetry and conservation laws.

Throughout, the presentation is orderly, with excellent summaries at the end of each chapter. Mathematical rigour is not pursued to lengths which might deter the physicists for whom the book is intended; but the treatment is far from anti-mathematical, and the author's balance between rigour and vigour is struck very skilfully. A substantial number of well chosen questions and problems occurs throughout the text, and they form an essential part of the development. Adopted as an expedient to reduce the book's length, this does not seem altogether satisfactory, and one is tempted to ask for the inclusion (in the next edition) of an appendix containing answers to the questions and notes on the problems: this would be particularly useful to readers studying the text without the support of a lecture course.

Richard M. Sillitto

R. M. Sillitto is Senior Lecturer in Physics at the University of Edinburgh, Scotland.

Nuclear physics

SEVENTEEN volumes are now available in the *Oxford Physics Series* (Oxford University Press: Oxford) edited by E. J. Burge D. J. E. Ingram and J. A. D. Matthew, and they already fulfil part of the declared aim of covering "in a flexible way, the material required for degree courses in pure physics, or physics combined with other subjects". The series as a whole is closely integrated, and Professor E. J. Burge's book, *Atomic Nuclei and their Particles* (hardback £6; paperback £3.25), together with that of G. K. T. Conn on *Atoms and their Structure* (forthcoming), provides a comprehensive and readable introduction to atomic and nuclear physics for first and beginning second-year undergraduates. Together with D. J. E. Ingram's book on *Radiation and Quantum Physics* (hardback £2.90; paperback £1.45) they provide the basis for G. A. Jones' more advanced text on *The Properties of Nuclei* (hardback £5.95).

Burge's book begins with an elementary discussion of atoms, electrons and photons, and reviews the early history of the development of nuclear physics, with chapters on the nucleus revealed by alpha particles, and nuclear accelerators and instruments. The remainder of the book is devoted to nuclear reactions, nuclear forces and models, cosmic rays, and elementary particles. It is clearly and skilfully written with just the right amount of detail, and is enlivened by historical remarks that will help to bring the subject alive to the reader.

After mastering Burge, students will be well prepared to tackle the more advanced text of G. A. Jones, written mainly for second- and third-year undergraduates. It begins with a general survey of nuclear properties, and goes on to consider nuclear forces and nuclear models in some detail. Chapters follow on the spontaneous decay of nuclei, in particular alpha and beta decay and electromagnetic transitions, and on nuclear reactions, fission and fusion. There are useful appendices on cross-sections, pairing, exchange forces and spin matrices.

The difficulties of treating these subjects at this level are overcome very successfully by judicious use of simplified calculations, illuminated by penetrating comments revealing a deep understanding of the subject. Indeed there are few professional nuclear physicists who would not find it a rewarding experience to read this book.

Taken together, these two books complement each other admirably and integrate well with the other books in the series. They are well equipped with problems for students to test their understanding of the subject. They deserve a warm welcome and should be widely used in physics courses in universities and other centres of higher education.

A word should be added on the method of production. Previous books in the series have been type-set and printed and the results are fully up to the high standards of the Oxford University Press. The books of Burge and Jones, however, are produced by reproduction from typescript. Although this is apparently necessary for reasons of economy, it must be said with regret that the overall effect is much less pleasing to the eye, making the books less easy to read. The lines are not justified or always evenly spaced; bad

breaks abound; there are fewer words per page; mathematical formulae are particularly clumsy; and the bubble chamber photograph on page 90 of Burge's book is illegible. The Oxford University Press deserves to be judged by the highest standards, and it is to be hoped that they will soon find ways of production that are both economical and worthy of their distinguished tradition. **P. E. Hodgson**

P. E. Hodgson is Lecturer in Nuclear Physics at the University of Oxford, UK.

Semiconductor devices

The Physics of Semiconductor Devices. By D. A. Fraser. Pp. 148. (Oxford University Press: Oxford, London and New York, 1977.) £5.95.

SEMICONDUCTOR DEVICES are made to operate by the use of a very wide range of sophisticated electrical tricks. Therefore, the task of compressing a treatment of the physics of the effects so used into a slim volume requires great skill in selection; and to make it easy to read and entertaining is even more of a challenge. Future generations of students will, I believe, say that Mr Fraser has met all of these challenges remarkably well at a level which will suit second-year degree students in physics or engineering. The book will also help people like myself, who have learnt solid-state physics in disconnected segments and wish to bring these pieces together. The fact that the book is one of a teaching series (*Oxford Physics Series*) and will relate well to other members of this series on the solid-state and electrical circuits, will help all classes of learner.

The subject-matter covered is about the same as that treated in earlier textbooks by Grove (370 pages) or Sze (800 pages) and can be divided up into about 60 pages on basic semiconductor physics and simple device theory and 60 on more advanced device theory and the discussion of modern technology and its implications. More attention is paid here than in the books mentioned above to teaching, in the sense of noting possible difficulties facing the student and taking pains to clarify these. The informal but highly informative style in which this is done is worth many pages of equations. Apart from a well-conceived figure giving an impression of the transport of an electron through a solid lattice, including scattering at centres and interaction with phonons, none of the diagrams were very original, which was a disappointment considering the authors expressed predilection for pictorial explanations. The art editor has also made things harder by using lettering which sometimes fades badly under the lithographic reproduction process used.

The technical evaluation of such a brief summary must, of course, turn on what was left out as much as what was included. Let us start by saying that all of the derivations of semiconductor equations which were included were very skilfully stripped down to their essentials, with useful suggestions as to how the student can bridge any of the reasonably-sized jumps from point to point. I would have only a few quibbles, such as the use of W for the potential energy of an electron and the showing of a complementary MOS circuit with an n-type well rather than the nearly universal p-type well.

The only omission which I felt to be considerable, even on the present scale, was the usual expression for common-emitter forward gain of a bipolar transistor in terms of emitter efficiency and of the two important forms of recombination—surface and depletion-region. The Ebers-Moll model of the transistor is presented very convincingly, followed by the hybrid- π representation, in a form which does not distinguish surface and bulk recombination. This may have been chosen because the author chose to deal with real bulk recombination effects later, when dealing with thyristors. He also notes, in a later section on surfaces, that he will avoid discussion of real surface states. My personal opinion is that surface recombination is a bit too important to omit from any book dealing with technology of as well as the basics of semiconductor physics. What is more, the bipolar transistor, quite rightly, is treated as the core of the book and hence could be used nicely as the door to all of the technological problems discussed later on. This is not to say that the treatment given to the bipolar transistor is not, as far as it goes, illuminating.

This is a book which, because of its free-flowing, simplified treatment of some rather stiff physical principles, will help many students to overcome their difficulties with solid-state physics and give them the confidence to derive their own simple equations before forging on to the complexities of electronic technology.

Andrew Holmes-Siedle

Andrew Holmes-Siedle is a consultant to the Physics Department of Fulmer Research Institute and to the European Space Agency, on nuclear radiation techniques, solid-state physics and space technology.

Mechanics of fluids

Physical Fluid Dynamics. By D. J. Tritton. Pp. 362. (Van Nostrand Reinhold: New York, 1977.) Paperback £5.50.

IN principle the mechanics of simple fluids, such as air or water, should be comprehensible; the equations which govern their behaviour are well established and there is a wealth of equipment, some of great sophistication, available for the study of the macroscopic phenomena. Nevertheless, such is the complexity of motions that many of their central characteristics are still very imperfectly understood, and consequently the subject proves enormously attractive to a wide range of scientists.

The subject can be taught from various viewpoints—mathematical or engineering are the commonest—each requiring a different emphasis, although its interdisciplinary property should always be borne in mind. Introductory textbooks are available in plenty to meet in part these special needs but the present book is rather unusual in that it is directed towards physicists who are more concerned with understanding the phenomena

without subjecting themselves to the full discipline of mathematical argument. It should, however, be of interest to all beginning students because of the wide sweep of topics in each of which a number of interesting, sometimes curious and even spectacular properties are described, usually with explanations using simple mathematical or physical ideas which stimulate the reader to find out more.

The author also recognises at once the central characteristic of fluid motions, namely, their proneness to instability and turbulence. He opens with a discussion of three examples—pipe flow, bluff bodies and convection—before deriving the equations of motion and later devotes about a third of the book to a general discussion of this characteristic. It is disappointing that most of the available knowledge is phenomenological and he sadly concludes that the practical engineer uses empirical methods which have “little support from fundamental studies”.

After a derivation of the Navier-Stokes equations, which is good physically but mathematically superficial, he then goes on to discuss a number of crucial concepts, vorticity, Reynolds number, inviscid flows, boundary layers and lift,

with the aim of giving the student a feel for fluid motions and an appreciation of major difficulties hampering progress. Convective processes are then discussed in more detail largely from physical and dimensional points of view, followed by useful accounts of the special features of rotating and stratified flow, for example, blocking, Taylor columns and internal waves. The book ends with comments on a number of important practical situations where the ideas of fluid mechanics are of significance.

While I felt uncomfortable at some points in the text, where the explanations offered seemed as mysterious as the phenomenon, and important concepts, such as group velocity, were given inadequate treatment, the competence, range of material and infectious enthusiasm permeating the book encourage me to recommend it to mathematical and engineering students as essential general reading.

K. Stewartson

K. Stewartson is Joint Head of the Department of Mathematics at University College, London, UK.

Relativity made simple

Tensors, Relativity and Cosmology. By Eric A. Lord. Pp. 207. (McGraw-Hill: London, New York and Toronto, 1977.) £3.30.

THERE is nothing especially new in this book, but no claim is made that there is. It is essentially a small, cheap, straight-to-the-point, teaching textbook on relativity theory, and I like it. The reverse side of the title page contains the comment: “This book has been subsidised by the Government of India through the National Book Trust, India, for the benefit of the students”, which can’t be bad. For only £3.30 it is certain to reach its core market, which is the mathematically inclined postgraduate student with no previous knowledge of relativity.

The book is short—just 200 pages—and necessarily concise. It is divided in an obvious way into three parts: tensors (19 pages), special relativity (61 pages) and general relativity (forming the bulk of the book with 111 pages). The whole thing is broken up into no less than 15 chapters, which if nothing else makes it easily digestible to the uninitiated readers. If part I is incomprehensible, the author refers us to part II.

There are, of course, quite a number of books at about this level, but *Tensors, Relativity and Cosmology* does have some distinguishing features to recommend it. First, it is more mathematical than many of its rivals. The treatment of tensors and differential geometry is traditional, but there is also a chapter on

gravitation and spinors. Second, several topics are treated which do not often crop up in books as narrowly directed as this. For example, a whole chapter is devoted to unified field theories and another to scalar-tensor theories.

Inevitably in a book that covers so many topics in such a short space, depth is sacrificed to comprehensiveness. The inclusion of cosmology in the title is not really fair, as it only warrants one chapter of 18 pages, and goes little beyond what can be found in, for example, Bondi’s original book. Only the standard Robertson-Walker models are discussed, with the steady-state theory packed into a couple of pages for good measure.

I doubt if the author’s claim that those who have mastered this book will be equipped to “carry out research in relativity and related topics” can really be justified. However, such a reader will certainly have a whetted appetite, and will wish to engage the more detailed textbooks for some of the topics only touched on here.

There will always be a market for books of this type that actually write down in the simplest possible way many of the things that have to be dug so painfully from the more pedantic works. There is the danger that it degenerates into a dry run-down of superficial facts, but I at least am pleased to have such a large amount of scattered information collected in one place. In short, a very convenient, if unexceptional book.

Paul Davies

Paul Davies is Lecturer in Mathematics at King’s College, University of London, UK.

BRITISH MUSEUM (NATURAL HISTORY) BOOKSHOP

All BM(NH) books and publications can be seen at the new bookshop in the Natural History Museum at South Kensington. The scientific works have now been placed in a section of their own where visitors can browse at leisure.

Students, teachers and researchers alike will find items of interest among hundreds of titles on biology, geology and mineralogy.

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Astrotexts updated

THERE are an enormous number of astronomy textbooks in print at the present time. However, if this number is divided up according to the age group and ability level to be served, we find that nearly half are aimed at university students with considerable knowledge and ability in associated subjects like mathematics, physics, chemistry and geology; and nearly half are aimed at the same 18–24 yr age group but this time assuming that the group has extremely limited knowledge of the advantages that mathematical expertise bestow on a person exploring the sciences and also scant knowledge of the experimental and observing techniques fundamental to all scientific enquiries. The remaining small percentage are aimed at that enormous group of younger, less academically minded scholars that fill our schools and don't go on to university afterwards.

An extremely valuable addition to this small percentage is *A Universe to Explore* (Holt-Saunders: Eastbourne, UK, £3.50) by Ralph O. Ewers and Lynda M. Ewers. This Canadian school-booklet consists mainly of a series of 'activities', some designed for use in the classroom with pencil and paper (for example, making a model to help understand the Ptolemaic and Copernican theories of planetary motion and to predict the phases of Venus, investigating how Cepheid luminosity is related to period), some in the class laboratory using simple equipment (constructing a simple telescope with two convex lenses, using a diffraction grating as a spectroscope, observing the phenomena of parallax for nearby objects and using this to measure distance) and some outside looking at the sky with the eye, telescope or camera (constructing and using a visual magnitude scale, observing planetary movement, sunspots and galaxies, and so on). The book abounds with activities, the main emphasis being on the scholars finding out for themselves. It has a fund of excellent ideas and will be an absolute godsend to school teachers entrusted with science education. The book is beautifully produced and colourfully illustrated and can be recommended most strongly. Even though it is firmly intended for young schoolchildren (under fifteen), university teachers will find many interesting ideas there, ideas that can be easily expanded to provide excellent practical experiments.

Staying with our young explorers of the universe and their teachers let me say a few words about *Astronomy: A Dictionary of Space and the Universe* by Iain Nicolson (Arrow Books: London, paperback £1.75). Publishers are always keen to jump on bandwagons and astronomical

dictionaries seem to be the bandwagon of the moment. Nicolson's has one definite advantage over many of the others, its price, but this is far from all that can be said of it. The author has included over 600 entries which explain clearly and succinctly the terms being discussed. There is an excellent system of cross referencing so that statements need not be repeated. The figures are a joy and receive a rightful and prominent place. Terms such as transit, culmination, spectroscopic binary, aberration of light and eclipse are illustrated with telling clarity. The book has 16 pages of plates and a most useful list of articles in a guide to further reading. No school teacher has an excuse now for astronomical ignorance, all he needs is this book in his pocket.

Moving now to our large fraction of innumerate students we have *Astronomy: The Structure of the Universe* by William J. Kaufmann III (Collier-Macmillan: London, £9.75). This book is designed for the one-semester general astronomy course taken by American liberal arts students, as are many other similar books. Kaufmann's has one interesting and refreshing change of emphasis, summed up by his statement that "Modern astronomy begins at distances greater than 1 light year from the Earth". So much for the Sun and planets I thought. Well not quite, but Kaufmann only spends 9% of his book on our celestial neighbours. What remains is a fascinating account of stellar and galactic astronomy with a special emphasis on topics of major interest to the amateur astronomer and interested students: pulsars, quasars, black holes, stellar evolution and cosmology. Instead of skirting round the topics of the special theory of relativity and the general theory of relativity, Kaufmann tackles them head on and devotes whole chapters to each. This book is a worthy addition to the groaning shelf of books aimed at this market; in fact it is more than just worthy addition, its different approach puts it ahead of many. Variety, the spice of life, will make many teachers pick this one if only to get away from the all too familiar plod through the normal list of planetary, stellar and galactic objects.

Focus on the Stars (Heinemann Educational: London, £4.80) is a collection of lectures, edited by H. Messel and S. T. Butler, and given at the sixteenth International Science School for High School Students at the University of Sydney. Bart J. Bok probes our Galaxy, and J. P. Wild has a close look at the Sun, concentrating mainly on its radio image. Carl Sagan views Mars with Mariner 9, R. Hanbury Brown introduces astrophysics and stellar interferometers, R. D. Brown looks at molecules in space, Peter Goldreich the evolution of the Universe and Frank D. Drake the radio reach for intelligent extraterrestrial life. This is a

most interesting set of lectures geared mainly for scholars with a scientific background. Its main drawback is that the lectures are old, most having been written in late 1972, early 1973. It's a great pity those responsible couldn't have sprung into action about four years sooner.

Let us end with two new editions of old favourites. *Textbook on Spherical Astronomy* by W. M. Smart (Cambridge University: London and New York, hardback £11.50; paperback £5.50) has been revised by R. M. Green and now comes out in its sixth edition. The first edition was published in 1931. Few astronomers would wish to find themselves too far away from a copy of this book, but it must also be said that few would regard it as light bedside reading matter. It is still a superb repository of the wonders and complexities of spherical astronomy with chapters on such topics as refraction, meridian circles, planetary motion, time, aberration, parallax, proper motions, precession, binary star orbits and eclipses. Green has revised the book so that its terminology is compatible with that of the *Astronomical Ephemeris* and the *Explanatory Supplement to the Astronomical Ephemeris* and the *American Ephemeris* and *Nautical Almanac*. There seems to be little in this new edition which will make owners of old editions throw them away and rush eagerly to the bookshop claspings £11.50.

Essentials of Astronomy by Lloyd Motz and Anneta Duveen (Columbia University Press: New York, \$21.90) reaches its second edition eleven years after the first. Much has changed in this time: lunar and planetary surfaces have been scanned; studies of stellar evolution, interstellar medium and interstellar molecules have progressed; neutron stars, pulsars, X-ray sources, black holes and quasars have all become big talking points. Most of the new material has been discussed in a 52-page section at the end of the book entitled "Developments in Astronomy Since 1966". This method of revision completely breaks up the continuity of the book, the reader continually having to dodge backwards and forwards to ensure he's not missing any new developments. Considering the price of the new book, the enormous popularity of the first edition and the obvious large sales that this new edition will have, I think the least the authors and publishers could have done was to revise it properly by incorporating the revisions into the text. However, putting this criticism to one side 'Motz and Duveen' still remains the cornerstone of many university first-year science courses in astronomy; it still retains its position as the best textbook in its class.

David W. Hughes

David W. Hughes is Lecturer in the Department of Astronomy and Physics at the University of Sheffield, UK.